Re: Fisher et al
Filed: 4/15/86

853404 X 3

STATE OF ISRAEL

זאת לתעודה כי
רצופים בזה העתקים
נכונים של המסמכים
שהופקדו לכתחילה
עם הבקשה לפטנט
לפי הפרטים הרשומים
בעמוד הראשון של
הנספח.

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particulars of which are
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This 12 II 1986 היום

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חוק הפטנטים, תשכ"ז — 1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

C: 03185

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH
POB 19
70 450 NES ZIONA.

המכון למחקר ביאולוגי בישראל
ת.ד. 19
נס-ציונה 70 450

The inventors: Abraham Fisher
Ishai Karton
Eliahu Heldman
Yona Grunfeld
Aharon Levy

הממציאים: אברהם פישר
ישי קרטון
אליהו הלדמן
יונה גרינפלד
אהרון לוי

בעל אמצאה מכח בדין
Owner, by virtue of by law

שמה הוא
of an invention the title of which is

(בעברית) נגזרות של קווינוקלידיין
(Hebrew)

(באנגלית) DERIVATIVES OF QUINUCLIDINE
(English)

מבקש בזאת כי ינתן לי עליה מסנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה רין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
No. מס'	No. מס'			
dated מיום	dated מיום			
• יפוי כח: כללי / מיוחד — מצורף / עוד יוגש P.O.A.: general / individual - attached / to be filed later-				
filed in case מוגש בענין				

כתובת למסירת מסמכים בישראל
Address for Service in Israel

Sanford T. Colb סנפורד ט. קולב
POB 2273 ת.ד. 2273
76 122 Rehovot רחובות 76 122

חתימת המבקש
Signature of Applicant

For the Applicant,

Sanford T. Colb

Patent Attorney & Advocate
C: 03185

היום 9 בחודש May שנת 1985
This 9 of May of the year 1985

לשימוש הלשכה
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סומס זה, כשהוא מוטבע בחותם לשכת הפטנטים ומושלם בספר יבתאריך התגשת, הנו אישור להגשת תבקשה שפרטיה רשומים לעיל
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• מחק את הסיותר Delete whatever is inapplicable •

Derivatives of Quinuclidine.

FIELD OF THE INVENTION

The present invention relates to novel spiro(1,3-oxathiolane-5,3')quinuclidines and a novel hydroxymercaptomethylquinuclidine; processes for preparing the novel compounds; pharmaceutical compositions containing the spiro-compounds; and a method for treating diseases of the central nervous system using such spiro-compounds or pharmaceutical compositions.

BACKGROUND OF THE INVENTION

US Patent No. 4,083,985 describes a range of fused-ring quinuclidines which are described as psychomotor stimulators, and which may be regarded structurally as a quinuclidine nucleus fused to a cyclohexanone, cyclohexenone or delta-lactone moiety. These compounds are stated to be useful for treating (inter alia) Parkinsons' Disease and depression, and evidently possess anticholinergic activity. There is no indication in this Patent that any of these compounds possess cholinergic activity.

US Patent No. 4,104,397 describes spiro(1,3-dioxolane-4,3')quinuclidines which may have one or two alkyl and/or aryl substituents in the 2-position of the dioxolane ring. The Patent specifically describes the monomethyl, dimethyl and diphenyl compounds. The monomethyl compound is shown to have cholinergic

activity and the diphenyl compound to have anticholinergic activity. The nature of the pharmacological activity exhibited by the other compounds embraced by this Patent is not described therein.

A chronic deficiency in vivo in central cholinergic function, that is to say in the function of acetylcholine as a neurotransmitter, has been implicated in a variety of neurologic and psychiatric disorders, including senile dementia of Alzheimer's type(SDAT), tardive dyskinesia, Pick's disease, Huntington's chorea, Gilles de la Tourette disease, Friedrich's ataxia, and Down's syndrome. Clinical data indicate that cholinergic transmission may have been compromised in persons affected with these diseases (Fisher and Hanin, Life Sciences, 27: 1615, 1980).

Among these disorders, SDAT is the most widespread neuropsychiatric disease (for reviews see Schneck et al, Am. J. Psychiatry, 139: 165, 1982 and Coyle et al, Science 219: 1184, 1983). The development of an effective treatment for SDAT is one of the most pressing needs facing medicine today. This age-related disease is becoming increasingly prevalent as the population of the elderly grows in line with the progressively higher life expectancy of the older population.

SDAT is characterized morphologically by an increased number of senile plaques in selected brain areas; biochemically by a significant reduction in presynaptic cholinergic markers in the same brain areas, the cortex and the hippocampus in

particular; and behaviorally by a loss of cognitive functions in individual patients.

Since SDAT appears to be associated with brain cholinergic hypofunction, trials have been conducted in which ACh precursors (choline or lecithin), acetylcholinesterase inhibitors (physostigmine or tetrahydroaminoacridine) or direct acting muscarinic agonists (arecoline) have been administered to SDAT patients because of the ability of these agents to elevate, and thus presumably restore cholinergic activity in the brain. To date, the results have not been conclusive as to the efficacy of treatment with the above-mentioned agents; this is due mainly to unwanted side-effects, narrow therapeutic window, or lack of therapeutic efficacy.

There is an urgent need for drugs which are effective in the treatment of SDAT. Progress in this area has been hindered by the lack of adequate animal models that can mimic directly the cholinergic abnormality implicated in SDAT, and by a dearth of long- acting central cholinergic agonists which can discriminate among subclasses of receptors, and primarily activate those that are involved in cognitive functions. Most known cholinergic agonists (muscarinic drugs) have undesirable side-effects. A long-lasting, centrally active cholinomimetic drug without peripheral side effects would therefore be most useful. The R & D of such drugs would require their evaluation in suitable animal models for SDAT.

In this context, we have recently developed a selective

presynaptic cholinergic neurotoxin, ethylcholine aziridinium ion (AF64A), which on intracerebroventricular injection in rats induces persistent cholinergic hypofunction that mimics the cortical and hippocampal cholinergic deficiency and the cognitive impairments reported in SDAT. This animal model could be extremely useful in developing novel treatment approaches for SDAT.(Fisher et al in Behavorial Models and the Analysis of Drug Action, eds. Spiegelstein and Levy, Elsevier, Amsterdam, 1983, p. 333; Fisher and Hanin, Ann. Rev. Pharmacol. Toxicol., 1985; in press).

The availability of centrally active muscarinic compounds which have long acting central cholinergic activity without significant peripheral adverse side-effects capable of reversing cognitive impairments induced by AF64A in rats, could be extremely useful in treating SDAT and the above-mentioned related disease states.

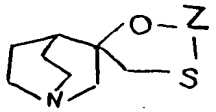
Therapeutically active oxathiolane compounds and their pharmacology are relatively little known. Moreover, the literature is replete with unsuccessful attempts to replace a particular atom or group in a pharmacologically active chemical compound by a supposedly analogous atom or group, in an attempt to improve the therapeutic profile of the original compound. Thus, in replacing for example an oxygen atom by a sulfur atom which has twice the atomic mass of oxygen, the result in pharmacological terms cannot be predicted with any degree of certainty.

However, we have now surprisingly discovered, and this discovery forms the basis of the present invention, that if in the spiro(dioxolane)quinuclidines of US Patent No. 4,104,397, the oxygen atom of the dioxolane ring which is more remote from the quinuclidine nucleus is replaced by a sulfur atom, and at the same time the ambit of the substituents at the 2-position is extended to include diarylmethylol, and alkyl substituted by aryl, then (i) the most active isomer of the monomethyl compound, while possessing a not dissimilar activity (measured by the Guinea-pig ileum induced contraction and muscarinic receptor binding tests) as compared with that of the most active isomer of the analogue disclosed in the aforementioned US Patent, however exhibits significantly less pronounced side-effects (sialogenic and tremorigenic activity) than the latter compound under similar conditions; and (ii) the most active isomer of the monomethyl compound has interesting potential for the treatment of SDAT as shown by the tests on animal models mentioned above.

On the other hand, the vast majority of the other 2-substituted members of the series containing the sulfur atom in place of oxygen as aforesaid, and in particular those members containing at least one 2-substituent which is alkyl having three or more carbon atoms, cyclopentyl, cyclohexyl, aryl, diarylmethylol or alkyl substituted by aryl, have anticholinergic activity, as contrasted with the cholinergic activity of the monomethyl compound.

SUMMARY OF THE INVENTION

The invention accordingly provides quinuclidine derivatives having the general formula (I)



and geometrical isomers, enantiomers, diastereoisomers, racemates and/or acid addition salts thereof, wherein Z represents the group $>CR^1R^2$ or two hydrogen atoms; and R^1 and R^2, which may be identical or different, are each alkyl, cyclopentyl, cyclohexyl, aryl, or diarylmethylol, or alkyl which is substituted by one or more aryl groups, or one of R^1 and R^2 may be hydrogen.

In accordance with one embodiment of the invention, Z in formula (I) represents the group $>CR^1R^2$, one of R^1 and R^2 is hydrogen, and the other of R^1 and R^2 is alkyl, cyclopentyl, cyclohexyl, aryl, or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

In accordance with a further embodiment of the invention, Z in formula (I) represents the group $>CR^1R^2$, one of R^1 and R^2 is alkyl, cyclopentyl or cyclohexyl, and the other of R^1 and R^2 is alkyl, cyclopentyl, cyclohexyl, aryl, or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

In accordance with still a further embodiment of the invention, Z in formula (I) represents the group $>CR^1R^2$, one of

R^1 and R^2 is aryl, and the other of R^1 and R^2 is aryl or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

When Z in formula (I) represents the group $>CR^1R^2$, the compounds thus defined have central nervous system activity. These compounds may be named as 2,2-disubstituted spiro(1,3-oxathiolane-5,3')quinuclidines. Non-limiting examples of such compounds are described in the following table:

one of R^1 and R^2	the other of R^1 and R^2	
hydrogen	methyl	(Ia)
hydrogen	ethyl	
hydrogen	propyl	
hydrogen	phenyl	
hydrogen	1-pyrenepropyl	
hydrogen	diphenylmethyl	(Ib)
hydrogen	diphenylmethylol	
methyl	phenyl	(Ic)
ethyl	phenyl	
cyclohexyl	phenyl	
phenyl	phenyl	

The invention also includes the compound of formula (I) wherein Z represents two hydrogen atoms, i.e. 3-hydroxy-3-mercaptomethylquinuclidine, from which the compounds of the invention in which Z in formula (I) represents the group $>CR^1R^2$ may be prepared.

As previously indicated, the invention includes the geometrical isomers, enantiomers, diastereoisomers, racemates and/or acid addition salts, of the compounds of formula (I).

It will be appreciated that geometrical isomerism arises from the fact that in the spiro-compounds of the

invention, R^1 for example, may be either on the same side of the oxathiolane ring as the nitrogen atom of the quinuclidine ring, or on the opposite side. If, in the spiro-compounds of the invention, R^1 and R^2 are identical, then there will be a single centre of asymmetry at the 5,3'(spiro) carbon atom, this will also give rise to enantiomers and the racemates thereof. If, on the other hand, R^1 and R^2 in the spiro-compounds are not identical, then there will be a further centre of asymmetry at the 2-position of the oxathiolane ring, thus giving rise to diastereoisomers and the racemates thereof, in addition to the geometrical isomers already mentioned. It will moreover be appreciated, that in the compound of the present invention which is 3-hydroxy-3-mercaptomethylquinuclidine, there exists no possibility of geometrical isomerism; here also, however, there is a centre of asymmetry at the 3-position of the quinuclidine ring, thus giving rise to enantiomeric and racemic forms.

The compounds of formula (I), whether as isomeric mixtures or compounds, or whether as individually isolated geometric or optical isomers, form stable addition salts with organic or inorganic acids, as for example with hydrochloric acid. It will be observed that, while for therapeutic application such salts should be pharmaceutically compatible, nevertheless it may be convenient, as for example for purposes of isolation, to employ acid addition salts which are not pharmaceutically compatible, and the invention relates also to the acid addition salts of the latter kind. As will be obvious to those skilled in the art, if the compounds are obtained e.g.

as a result of isolation from their process of preparation in the form of free bases, they may be converted to the acid addition salts by reaction with the appropriate acid, and conversely, the compounds isolated in the form of their acid addition salts may be converted by reaction with a base, such as an alkali metal hydroxide, to the corresponding free bases.

Geometrical isomers are generally isolated by a physical method such as fractional crystallization (of the isomers per se, or of their salts) or column chromatography (using high or low pressure liquid chromatography techniques), while optical isomers are isolated by forming a salt mixture with an optically active complementary reagent(in the present case an optically active acid), followed by fractionation of the mixture, and isolation of the desired optical isomers from the salt fractions.

In a particular embodiment, the invention provides the isolated geometrical isomers of the compound wherein one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is methyl(Ia). These isomers may be differentiated from each other by the fact that their salts with hydrochloric acid have quite distinct relatively higher and relatively lower melting-points. The hydrochloric acid salt of the mixture of geometrical isomers of compound (Ia) which is isolated from the preparative process of the invention, as well as the hydrochloric acid salts of the individual geometrical isomers are also included within the scope of the invention.

In accordance with the invention, the compounds of formula (I) wherein Z represents the group $>CR^1R^2$, are prepared by a process which comprises reacting 3-hydroxy-3-mercaptomethylquinuclidine with a ketone of formula R^1-CO-R^2, and isolating the desired product from the reaction mixture. The process is desirably carried out in the presence of an acid catalyst, such as a Lewis acid, as for example boron trifluoride, which compound may be conveniently used in the form of its complex with diethyl ether, otherwise known as boron trifluoride etherate.

The process is also preferably carried out in presence of an inert organic solvent medium, as for example, chloroform or dichloromethane. The temperature at which the process of the invention is effected is not critical, but it will evidently be advantageous to use as low a temperature as is consistent with a reasonable yield, in order to avoid contamination of the desired product with byproducts which could result from decomposition and/or side reactions at higher temperatures. It is found that when conducting the process of the invention in presence of a catalyst such as boron trifluoride etherate, a temperature within the range of about 20 to 30 °C is suitable, but higher or lower temperatures may of course be used; a reaction temperature of about 25 °C is preferred. In order also to avoid undesirable contamination due to oxidation, it is preferable to conduct the reaction in an atmosphere of inert gas such as nitrogen.

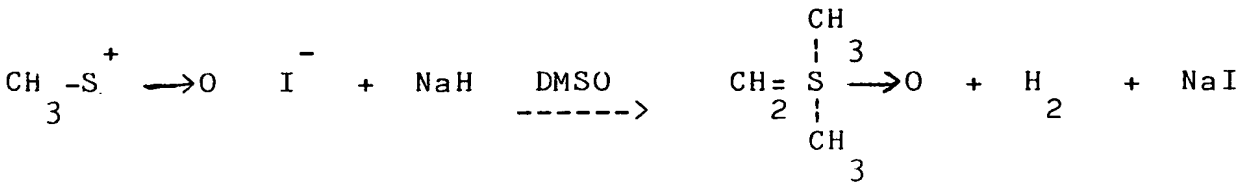
In a particular embodiment of the invention, therefore,

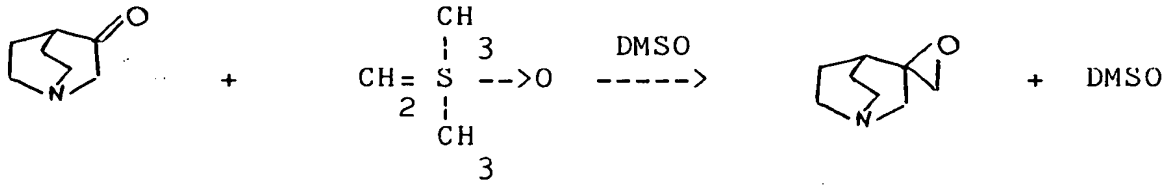
a process for preparing the compounds of formula (I) wherein Z represents the group $>CR^1R^2$, comprises reacting 3-hydroxy-3-mercaptopropylquinuclidine with a ketone of formula R^1-CO-R^2, in an atmosphere of nitrogen, at a temperature in the range of about 20 to about 30 °C, preferably at about 25 °C, in the presence of boron trifluoride etherate as catalyst and in dichloromethane as the solvent medium, and isolating the desired product from the reaction mixture.

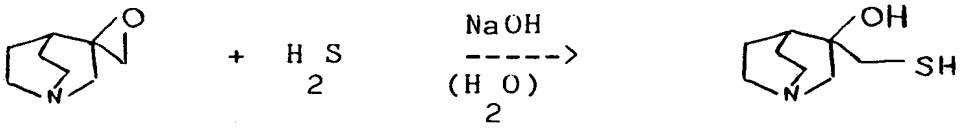
In this particular embodiment of the process, it is preferred that the reaction ingredients are first mixed in an atmosphe of nitrogen at a temperature between about -10 and +20 °C, e.g. at about 0 °C and the mixture thus obtained is permitted to rise to the reaction temperature.

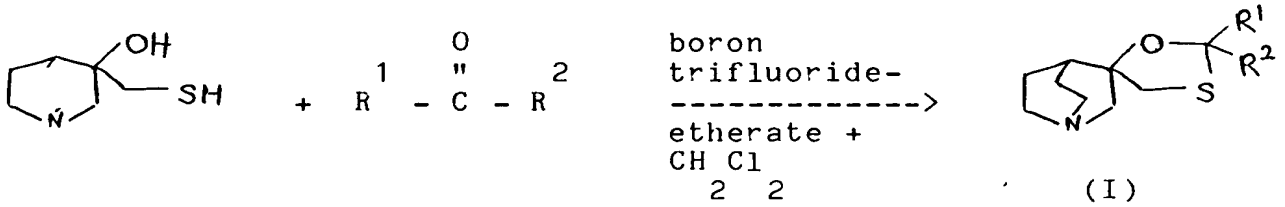
The invention further provides a process for preparing the compound of formula (I) which is 3-hydroxy-3-mercaptomethylquinuclidine, wherein the epoxide of 3-methylenequinuclidine is reacted with hydrogen sulfide, preferably in the presence of a base such as sodium hydroxide, more preferably in an aqueous medium. The epoxide may itself be prepared by reacting quinuclidin-3-one with dimethylsulfoxonium methylide.

The preparative processes of the present invention, including the processes by which relevant starting materials are prepared, are illustrated in a preferred embodiment, in the following reaction scheme:









(DMSO =dimethylsulfoxide)

REACTION SCHEME

The spiro-compounds of formula (I) have central nervous system activity. Thus, for example, the compound (Ia) is a muscarinic agonist with a high specificity for the central nervous system. Due to its pharmacological properties, it can be used to activate central cholinergic functions under conditions where the cholinergic system is hypofunctional. It can accordingly be utilized for the treatment of conditions such as senile dementia of Alzheimer's type(SDAT), tardive dyskinesia, Pick's disease, Huntington's chorea, Gilles de la Tourette disease, Friedrich's ataxia, and Down's syndrome, because all of these are disturbances in which a central cholinergic hypofunction has been implicated to some extent. This compound appears to be of especial value for the treatment of SDAT, since it is effective in reversing memory disorders due to AF64A-induced cholinotoxicity in a suitable animal model for this disease. In particular, the geometrical isomer of compound (Ia), of which the hydrochloric acid salt has the relatively lower melting-point, and which has been assigned the code number AF102B, reverses memory impairments in AF64A-treated rats as shown in a passive avoidance test and in the Morris Swimming Maze (see Morris, Learning and Motivation, 12: 239-61, 1978). [The other geometrical isomer of the compound (Ia), of which the hydrochloric acid salt has the relatively higher melting-point, has been assigned the code number AF102A]. In this context, AF64A (3 nmol/2ul/side, icv) induces marked cognitive impairments in a step-through passive avoidance test and in the Morris

Swimming Maze when the rats are analyzed four to eight weeks following treatment (Brandeis et al, in Alzheimer's and Parkinson's Disease: Strategies in Research and Development, eds. Fisher et al, Plenum Press, New York, in press; Fisher and Hanin, Ann. Rev. Pharmacol. Toxicol., 1985, in press).

The beneficial effects of AF102B in both memory tests occur at low doses (0.1 - 1 mg/kg, ip) and the therapeutic index is 50-500. This therapeutic index is wider than that found for physostigmine (5-17). Moreover, the slope of the acute toxicity curve is very steep, and no overt behavioral effects including parasympathomimetic effects, such as salivation or tremors, were detected up to the lethal dose. In this regard, the compound is superior to well known muscarinic agonists including arecoline and oxotremorine, in which such adverse side-effects complicate their possible use in SDAT therapy. In addition, AF102B has a long duration of action in the above-mentioned memory tests.

In SDAT patients, AF102B can be used in combination with anticholinesterase inhibitors such as physostigmine or tetrahydroaminoacridine; in combination with acetylcholine precursors such as choline or lecithin; in addition to "nootropic" drugs such as piracetam, aniracetam, oxiracetam or pramiracetam; in addition to compounds that interact with Ca^{2+} channels such as 4-aminopyridine or 3,4-diaminopyridine; or in addition to peptides that can have modulatory effects on acetyl choline release, such as somatostatin.

AF102B, with or without the aforementioned other active substances, can be administered for example, by way of injection in a suitable diluent or carrier, per os, rectally in the form of suppositories, by way of insufflation, by infusion or transdermally in a suitable vehicle with or without physostigmine, for example by using the device which is the subject of Israel Patent Application No. 72684 (vide infra). This compound may also be used in disturbances where cholinergic underactivity is induced by drugs.

The compound (Ia), in the form of either geometrical isomer, or a mixture of such isomers, is also of use for the treatment of disorders requiring the application of a long-lasting cholinergic agent of mild local activity. Such an agent is needed in disorders such as glaucoma, as the compound is not destroyed by the enzyme which deactivates acetylcholine, i.e. acetyl- and butyryl-cholinesterase. This compound may also be used for the treatment of peripheral cholinergic disorders such as myasthenia gravis, urinary bladder dysfunctions, Adi's disease and Eaton-Lambret disease.

When in the spiro-compounds of formula (I), R^1 and/or R^2 are propyl or higher alkyl groups, cyclopentyl, cyclohexyl, aryl, diarylmethylol, or alkyl substituted by aryl, the nature of the pharmacological activity of these compounds is changing, insofar as while they still possess central nervous system activity, this activity is now becoming anticholinergic instead of cholinergic. Such anticholinergic compounds can be used to treat disorders due to cholinergic hyperfunction, whether this

be spontaneous or drug-induced. These compounds may accordingly be used in the treatment of Parkinson's disease, pseudo-Parkinson's disease, mental depression and as adjuncts of surgery instead of (e.g.) atropine or scopolamine. They may also be used in ophthalmology when prolonged mydriasis is required for diagnostic and therapeutical purposes.

Where the term "pharmaceutical composition" is used in the specification and claims, this is to be understood in the sense that it may be suitable for human and/or veterinary treatment.

According to a further aspect of the invention, therefore, there is provided a pharmaceutical composition which comprises a quinuclidine derivative of formula (I), wherein Z represents the group $>CR^1R^2$, or a pharmaceutically compatible acid addition salt thereof, together with an inert carrier or diluent. This composition may be in a form suitable for oral, rectal or parenteral administration, or for administration by insufflation, or in particular it may be in a form suitable for transdermal administration, and in any event the composition may be in unit dosage form.

The pharmaceutical composition may contain as the spiro-compound of formula (I), for example, the compound identified herein as (Ia), and in particular the geometrical isomer(AF102B) of which the hydrochloric acid salt has the relatively lower melting-point. For the reasons noted hereinbefore, such a composition may contain as a further

ingredient or ingredients, one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, aniracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin.

Alternatively, the pharmaceutical composition may contain as the spiro-compound of formula (I), such a compound in which one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl and alkyl substituted by aryl, and the other of R^1 and R^2 is as previously defined, and in particular this compound may be one of those identified herein as (Ib) and (Ic).

When the pharmaceutical composition is to be administered transdermally, it is preferred to utilize the drug delivery system according to Israel Patent Application No. 72684. Thus, there is also provided in accordance with the invention, a pharmaceutical composition for transdermal administration, which comprises a compound of formula (I) wherein Z represents the group $>CR^1R^2$, or a pharmaceutically compatible acid addition salt thereof, as well as a low molecular weight fatty acid.

The invention also relates to a method for treating diseases of the central nervous system in mammals(i.e. in humans or in non-human mammals), which comprises administering to the mammal a compound of formula (I) or a pharmaceutically compatible acid addition salt thereof, wherein Z represents the group $>CR^1R^2$, which compound may of course be administered in the form

of the pharmaceutical composition described hereinbefore. This method for treating diseases of the central nervous system in mammals may of course also be applied by using the drug delivery system of the Israel Patent Application which has been described above.

More specifically, the method for treating diseases of the central nervous system in mammals, in accordance with the present invention, may be used for treating diseases due to a deficiency in the central cholinergic system in mammals, in which case the method comprises administering to the mammal the compound (Ia), including the geometrical isomers, enantiomers, diastereoisomers, racemates and/or acid addition salts thereof; the compound (Ia) may be administered in the form of a pharmaceutical compositions, which may be optionally supported for transdermal administration in the form of the device of Israel Patent Application No. 72684, as has already been described hereinbefore.

In another aspect of the present invention, the method for treating diseases of the central nervous system in mammals may be used for treating diseases due to a cholinergic hyperfunction in mammals, in which case the method comprises administering to the mammal the spiro-compound of formula (I) in which one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl and alkyl substituted by aryl, and the other of R^1 and R^2 is as previously defined, as for example, either of the compounds (Ib) and (Ic), including the geometrical isomers,

enantiomers, diastereoisomers, racemates and/or acid addition salts of such spiro-compounds of formula (I); these compounds may be administered in the form of pharmaceutical compositions, which may be optionally supported for transdermal administration in the form of the device of Israel Patent Application No. 72684, as has already been described hereinbefore.

In yet a further aspect of the present invention, the method for treating diseases of the central nervous system may be used for treating senile dementia of Alzheimer's type in humans, in which case the method comprises administering to a patient the geometrical isomer of compound (Ia), the hydrochloric acid salt of which has the relatively lower melting-point, including the enantiomers, diastereoisomers, racemates and/or acid addition salts of this geometrical isomer; the geometrical isomer of the compound (Ia) may be administered in the form of pharmaceutical compositions, which may be optionally supported for transdermal administration in the form of the device of Israel Patent Application No. 72684, as has already been described hereinbefore. Optionally, there may be administered in general, together with the aforementioned geometrical isomer of the compound (Ia), one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, aniracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin. In the case of transdermal administration, however, the additional ingredient is preferably one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, 4-

aminopyridine and 3,4-diaminopyridine.

For the purpose of definition, it is intended that the expression "method for the treatment of diseases of the central nervous system", and like expressions, throughout the specification and claims, be taken to include a method for the prevention of drug-induced diseases of the central nervous system.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the NMR(250 MHz) spectrum of 3-hydroxy-3-mercaptomethylquinuclidine.

Fig. 2 shows the NMR(250 MHz) spectrum of cis:trans (Ia)-HCl salt [AF102].

Fig. 3 shows the NMR(250 MHz) spectrum of the geometrical isomer of (Ia)-HCl salt identified as AF102A.

Fig. 4 shows the NMR(250 MHz) spectrum of the geometrical isomer of (Ia)-HCl salt identified as AF102B.

Fig. 5 shows the mass spectrum of 3-hydroxy-3-mercaptomethylquinuclidine.

Fig. 6 shows initial and retention-test latency measures of AF64A and CSF-injected groups, before and after physostigmine or saline.

Fig. 7 shows initial and retention-test latency measures of AF64A and CSF-injected groups, before and after AF102B or saline.

Fig. 8 shows retention-test latency measurements for (inter alia) AF102B in extinction trials.

Fig. 9 shows retention-test latency measurements for (inter alia) AF102B in "extinction + latent extinction" trials.

Fig. 10 shows retention-test latency measures of AF64A and CSF-injected groups, after second administration of AF102B or saline.

Fig. 11 shows escape latency measures, in blocks of two trials, of AF64A and CSF-injected groups, after physostigmine administration.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in further detail with regard to Examples for the preparation of the compounds of the invention, and biological testing of the spiro-compounds of the invention.

EXAMPLE I

Preparation of 2-methylspiro(1,3-oxathiolane-5',3)quinuclidine. [AF102 (cis: trans); AF102A and AF102B]

(a) Epoxide of 3-methylenequinuclidine.

In a 3l. three-necked flask fitted with a mechanical stirrer and thermometer, was placed sodium hydride (42g., 0.88 mole, as a 50% dispersion in oil) and 300ml. petroleum ether (30-60°). The suspension was stirred, the hydride allowed to settle,

the petroleum ether decanted, and with stirring 1200ml. of dry DMSO was added, followed by trimethyloxosulfonium iodide (214g., 0.97 mole), which was added portionwise over a period of 15 minutes, and stirring was then continued for an additional 30 minutes. The reaction flask was equipped with a sealed pressure-compensating dropping funnel containing quinuclidin-3-one (100g., 0.8 mole) dissolved in 300ml. dry DMSO. This solution was then added to the reaction mixture over a 15 minute period. After stirring for 15 minutes, the reaction mixture was heated to 50°C for 2 hours, poured into 1l. of cold water and the mixture extracted with 3 x 500ml. portions of benzene. The combined extracts were washed with 100ml. of saturated aqueous salt solution, dried over anhydrous sodium sulfate, and the solvent evaporated. The oily residue was dissolved in ether and precipitated as the salt with hydrochloric acid by addition of ether saturated with gaseous HCl. The epoxide product was separated by filtration, washed with ether and dried to give 100g. product which was sufficiently pure to use for the next step. R_f 0.3 on neutral alumina (ethyl acetate); M^+ = 139 (mass spectra determinations on a VG 7035 instrument); HCl salt has m.p. 200.7 - 202°C.

(b) 3-hydroxy-3-mercaptomethylquinuclidine.

In a 1l. three-necked flask equipped with a magnetic stirrer, an inlet and an outlet for hydrogen sulfide and a thermometer, were placed 80g. NaOH and 390ml. water. The solution was cooled in an ice-bath and gaseous hydrogen sulfide was passed into the stirred solution until it began to bubble out. The product of step (a) (80g., 0.46 mole) was then added,

and stirring was continued for 15 minutes. The reaction mixture was heated to 45°C for 2.5 hours under a slow stream of hydrogen sulfide. The solution was cooled to 0°C, and 10N HCl was carefully added to bring the pH to 8; the aqueous solution was then extracted with chloroform (6 x 300ml.). The combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The resulting solid was dried over phosphorus pentoxide in a dessicator to give 32.9g. of crude product. After purification (TLC, neutral alumina, 10:1 dichloromethane/methanol) R_f 0.5; M^+ (see Fig. 5) = 173; base peak = 140; NMR (see Fig. 1) peak at 250MHz($CDCl_3$) 2.8 -> double doublet, $-CH_2-SH$, AB-type spectrum; $IR_{\nu max}$ 2900-3300 $cm.^{-1}$ (broad). The compound was used for the next step without any further purification.

(c) 2-methylspiro(1,3-oxathiolan-5,3')quinuclidine.[AF102 cis:trans]

In a 0.5l. three-necked flask equipped with a magnetic stirrer, an inlet and an outlet for nitrogen gas and a thermometer, were placed the product of step (b) (32g., 0.18 mole), 200ml. dichloromethane (dried over molecular sieves) and freshly distilled acetaldehyde (110ml., 1.97 mole). The solution was cooled in an ice-bath under nitrogen gas, and boron trifluoride etherate (60ml., 0.40 mole) was added over a period of 30 minutes. The mixture was stirred three hours at 25°C, cooled to 0°C, and then treated with 10% aqueous NaOH solution until it was alkaline to litmus. The alkaline solution was extracted with chloroform (4 x 400ml.), and the combined extracts

were dried over anhydrous sodium sulfate and evaporated. The oily residue was dissolved in ether and precipitated by addition of gaseous HCl. The product hydrochloride was separated by filtration, washed with ether and dried, to give 26.5g.(yield: 62%), as a mixture of two geometric isomers in a ratio of 0.8 - 1.2 :1. R_f (TLC, neutral alumina, chloroform) 0.7; IR ν_{max} (using a Perkin-Elmer 457 grating infrared spectrophotometer) C-O 1200-1250, 1150 cm.-1; M^+ = 199; high resolution molecular weight determination: calc. for $C_{10}H_{17}NOS$: 199.1020, found: 199.1017.

NMR of the HCl salt is shown in Fig. 2. Resolution of the spectrum enables the existence of both isomers to be seen. The location of the -S- in the oxothiolane ring is evident from the chemical shifts of the respective Ha and Hb protons. These protons in the isomeric mixture and also in each of the isomers appear at higher field than their possible chemical shifts if they had been attached to -O- as in the analogous 1,3-dioxolane structure.

The product can be recrystallized from ethyl acetate (600ml. per g.) or from acetone (220ml. per g.).

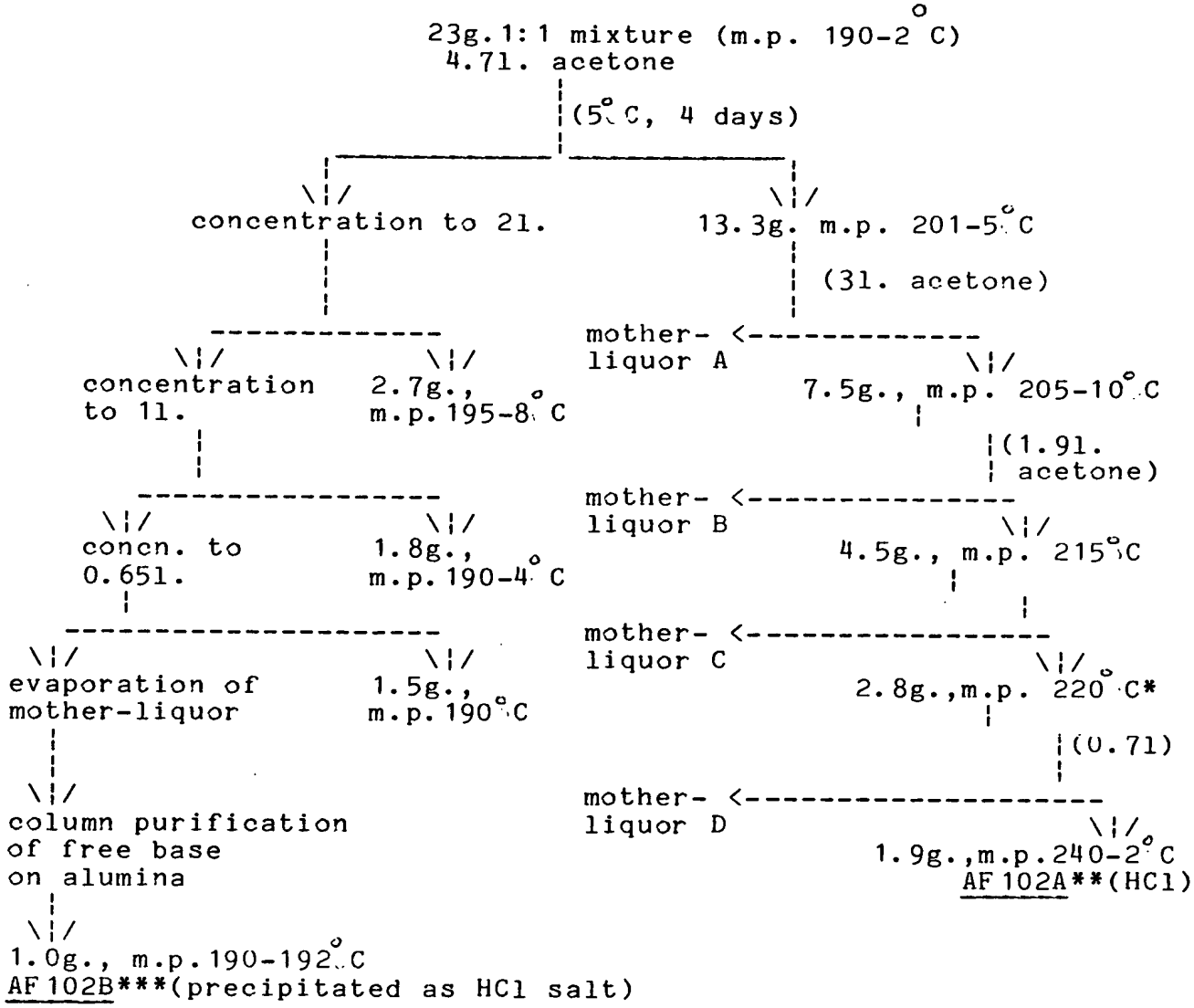
The isomeric mixture of hydrochlorides can be separated into its components by fractional recrystallization in redistilled dry acetone. Melting points as well as NMR spectra of 250 MHz can be used as an indication of purity for each isomer. Such a separation will now be described.

(d) Separation of AF102 into its components AF102A and AF102B.

A 1:1 isomeric mixture of hydrochlorides was crystallized from 4.7l. of redistilled dry acetone. The product

which precipitated was crystallized again (see Table), to yield after four crystallizations 1.9g. of pure AF102A, m.p. 240-242°C. Concentration of the mother liquor (see Table) resulted in an oily product which was purified on an alumina column(as a free base, 1% methanol in dichloromethane) to give mainly (10:1) the isomer AF102B, m.p. 190-192°C as the HCl salt.

TABLE: Separation of AF102A and AF102B as HCl salts.



* isomer ratio 10:1
** pure isomer (NMR)
*** isomer ratio about 1:10 (NMR)

AF102A(HCl): NMR (see Fig. 3), 250 MHz ($CDCl_3$) d 5.24 (quartet, peak of R^1 = H, which is a quartet since it is coupled to R^2 = CH_3).

AF102B(HCl): NMR (see Fig. 4), 250 MHz ($CDCl_3$) d 5.17 (quartet, peak of R^2 = H, which is a quartet since it is coupled to R^1 = CH_3).

EXAMPLE II

Preparation of 2-diphenylmethylspiro(1,3-oxathiolane-5,3')quinuclidine.

In a 100ml. three-necked flask equipped with a magnetic stirrer, an inlet and outlet for nitrogen and a thermometer, were placed 3-hydroxy-3-mercaptomethylquinuclidine (8g., 0.045 mole), 40ml. dichloromethane (dried over molecular sieves) and diphenylacetaldehyde (15ml., 0.085 mole). The solution was cooled in an ice bath, and kept under nitrogen while distilled boron trifluoride etherate (20ml., 0.13 mole) was added over a period of 30 minutes. The mixture was stirred two hours at 25°C, cooled to 0°C and then treated with 10% aqueous sodium hydroxide until the solution was alkaline to litmus. The basic solution was extracted with benzene (4 x 400ml.), and the combined extracts were dried over anhydrous sodium sulfate and evaporated. The oily residue was dissolved in ether and precipitated as the hydrochloric acid salt by addition of gaseous HCl. The product was further purified on a neutral alumina column as free base using 1:10 petroleum ether(40-60°)/ ethyl acetate as the eluent. Under these conditions, 2g. of the title compound were isolated. M^+ = 351.

EXAMPLE III

2-methyl-2-phenylspiro(1,3-oxathiolane-5,3')quinuclidine.

This compound was prepared by a similar method to Examples 1 and 2, by reacting acetophenone with 3-hydroxy-3-mercaptomethylquinuclidine. The yield was relatively low (10-20%). M^+ = 275.

Various other compounds within the ambit of formula (I) can be prepared by the exemplified method by using the appropriate aldehyde R^1-CHO or ketone R^1-CO-R^2, where R^1 and R^2 are as defined with respect to formula (I).

As has already been mentioned, the spiro-compounds of formula (I), including their pharmaceutically compatible acid addition salts may be formulated, together with conventional pharmaceutical inert carriers, diluents, adjuvants and so forth, as pharmaceutical compositions, which may be in a form suitable for oral, rectal or parenteral administration, or for administration by insufflation, or they may be in a form suitable for transdermal administration, and in any event the composition may be in unit dosage form.

The spiro-compounds of formula (I), or such pharmaceutical compositions containing them, may be administered to humans, as well as to laboratory animals such as rodents, cats, dogs and monkeys, as for example by peripheral or intracerebral or intracerebroventricular injections(for animals),

by infusion, through the skin, or per os. It is evident that the dosage and the route will need to be adjusted according to the specific biological use, and in particular according to the nature of the disease or disorder to be treated and its severity.

For the reasons which have already been noted hereinbefore, such pharmaceutical compositions may, in the case of (Ia), contain as an additional ingredient, one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, aniracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin.

As has also been previously mentioned in some detail above, when the pharmaceutical compositions are to be administered transdermally, it is preferred to utilize the drug delivery system according to Israel Patent Application No. 72684.

Biological Testing of the Spiro-Compounds of the Invention.

For AF102A and AF102B the general toxicity profile was first established. This study phase was routinely divided into two parts, i.e. "dose range finding" and LD_{50} determination. In the former, usually pairs of mice were administered widely spaced dosages and the animals were subsequently observed. The type, time of onset and duration of reactions to treatment were recorded. LD_{50} determinations were based on the preliminary findings of the range-finding studies. Under conditions of this

study, the test material solutions were administered to at least five dose-level groups of at least five animals of the same sex per group and at geometrically-spaced dosages. On the basis of mortality, the LD_{50} value and 95% confidence interval (C.I.) were calculated according to Weil's method (Weil, Biometrics, 8: 249-283, 1952).

AF102A

(a) LD_{50} mice; oral administration.

Test conditions - male mice, 20 - 24g.
- N = 5/group
- constant volume-dosage of 20ml./kg.

Dose (mg./kg. per os)	Mortality (number dead/number in group)
160	5/5
139	5/5
120	4/5
104	2/5
90	2/5
78	0/5

LD_{50} (95% C.I.) = 102 (89 - 117) mg./kg.

(b) LD_{50} mice; intravenous administration.

Test conditions - male mice, 20 - 24 g.
- N = 6/group
- variable volume-dosage of 9.0 - 11.5 ml./kg.

Dose (mg./kg. i.v.)	Mortality (number dead/number in group)
45.0	6/6
42.5	4/6
40.0	2/6
37.9	3/6
36.0	2/6

LD_{50} (95% C.I.) = 40.4 (35.5 - 45.9) mg./kg.

The relatively narrow ratio of about 1: 2.5 between the LD_{50} values of the i.v. and oral routes of administration suggests an efficient, rapid and apparently unaltered absorption of the test material by the enteric route.

(c) Signs observed.

Signs in reaction to treatment in mice and listed below were mostly confined to decedents, as survivors of treatment groups, administered respectively doses which caused only partial lethality, rarely demonstrated any of the reported affects in decedents. Undoubtedly, this finding points to a rather steep toxic-lethal slope of the test material.

Furthermore, there was essentially no obvious difference in effects observed between orally and intravenously treated animals, with the exception of time of onset, duration and time of death. Examination to assess pupillary changes were carried out only in surviving animals.

Signs are listed in order of their appearance:

- slight transient decrease of spontaneous motor activity (only in orally treated animals)
- severe tremors, particularly of head region (within 2-3 mins. or 10 secs. after oral or intravenous administration, respectively)
- dyspnoea
- convulsive seizures (clonic)
- gasping, cyanosis
- singular clonic-tonic convulsive seizure followed by death.

Death in mice to which were administered approximate LD_{50} doses, occurred about 10-15 mins. after oral administration and at about 30 secs. after intravenous injection, respectively. In surviving animals, transient partial analgesic activity in response to tail-pricking could be detected. Likewise, even among survivors in which one of the above-described signs was shown, full recovery was extremely rapid. Another feature worthwhile mentioning is the finding of slight to moderate mydriasis after pupillary examination, in surviving animals of the intravenously-treated group.

It should be pointed out that none of the test animals exhibited effects considered characteristic of parasympatho- or cholinomimetic activity, i.e. continuous generalized tremors, salivation, lachrymation, or diarrhoea and miosis.

Rats to which were administered an oral dose of 100mg./kg. showed the following:

- 10 mins. post-administration (p.a.), spontaneous motor activity was slightly reduced
- 15 mins. p.a. mydriasis, with pupillary size being about three times that seen in controls
- 20 mins. p.a., sudden onset of a brief clonic convulsive seizure which 5 mins. later changed into periodic head twitching and tremors
- 30 mins. p.a., animals appeared somnolent, accompanied by partial loss of righting reflex, dyspnoeic and cyanotic, with very slight salivation and lachrymation.

All of the above signs refer mostly to the one animal, out of the group of three, which was found dead at 40 mins. post-administration. In the remaining two rats, all of the above-displayed effects were much less in their relative intensity, and full recovery occurred after several hours.

AF102B

(a) LD_{50} mice; oral administration.

Test conditions - male mice, 20 - 24g.
- N = 5/group
- constant volume-dosage of 20ml./kg.

Dose (mg./kg. per os)	Mortality (number dead/number in group)
113	4/5
101	3/5
90	2/5
80	2/5
71	0/5
64	0/5

LD_{50} (95% C.I.) = 92 (74 - 116) mg./kg.

(b) LD_{50} mice; intravenous administration.

Test conditions - male mice, 20 - 24 g.
- N = 6/group
- variable volume-dosage of 9.0 - 11.5 ml./kg.

Dose (mg./kg. i.v.)	Mortality (number dead/number in group)
39	6/6
37	6/6
35	5/6
33	1/6
31	3/6

LD_{50} (95% C.I.) = 33 (31 - 35) mg./kg.

The relatively narrow ratio of about 1: 2.5 between the

LD_{50} values of the i.v. and oral routes of administration suggests an efficient, rapid and apparently unaltered absorption of the test material by the enteric route.

(c) Signs observed.

Inasmuch as both LD_{50} values after i.v. and per os administration of AF102B were slightly lower than those obtained with AF102A, the general signs of reaction to treatment with the former were essentially similar to those reported for the latter, with the mere difference being confined to a slightly enhanced intensity in the case of AF102B.

It should be pointed out that, as with AF102A, none of the test animals exhibited effects considered characteristic of parasympatho- or cholinomimetic activity, i.e. continuous generalized tremors, salivation, lachrymation, or diarrhoea and miosis. This feature is in contrast to the cholinergically active compound of US 4,104,397, namely cis-2-methylspiro(1,3-dioxolane-4,3')quinuclidine, hereinafter referred to under the code number AF30, which did exhibit such side effects.

TABLE 1. The potency of putative cholinergic compounds in displacing $\{^3H\}$-quinuclidinyl benzilate from central muscarinic receptors (whole rat brain excluding cerebellum and brain stem). Results are expressed as IC_{50}:

COMPOUND	IC_{50} {M}
oxotremorine	3×10^{-6}
AF102B	10^{-5}*
AF102A	7×10^{-5}

*approximately twice as potent as AF30

Muscarinic receptor assay was performed according to Yamamura and Snyder, PNAS US 71: 1725, 1974.

TABLE 2

Guinea-pig ileum induced contractions

COMPOUND	Type of activity	EC_{50} {M}	IC_{50} {M}*
Acetylcholine	agonist	5.0×10^{-8}	
AF30	agonist	4.0×10^{-6}	
AF102B	agonist	4.0×10^{-6}	
AF102A	agonist	$>10^{-5}$	
(Ib)	antagonist		$\sim 10^{-8}$
(Ic)	antagonist		$\sim 10^{-6}$

*This dose is the IC_{50} for inhibiting acetylcholine-induced contractions of the ileum.

From TABLES 1 and 2 it is evident that AF102B is a potent muscarinic agonist, whereas its geometrical isomer AF102A is almost one order of magnitude less active.

BEHAVIORAL STUDIES.

Summary of Results

Behavioral studies in rats treated icv with AF64A (3 nmole/2ul/side) revealed marked cognitive dysfunctions in a step-through passive avoidance test. These memory impairments could be reversed by physostigmine and by AF102B.

This reversal occurs at low doses of physostigmine (0.06 mg./kg., ip) and AF102B (0.1 and 1 mg./kg., ip). Therefore the "therapeutic index" of AF102B is 50-500. This "therapeutic index" is definitely wider than is found in physostigmine (5-17).

Moreover, it is important to note that the slope of the acute toxicity curve of AF102B is very steep and no overt behavioral signs including parasympathomimetic effects, such as salivation and tremors, were detected up to the lethal dose. Thus, the range of the "sign free" dose is rather wide, emphasizing the potential use of this drug for treatment of SDAT.

In addition, in the extinction studies performed in the AF64A-treated rats, a very long beneficial effect of AF102B was found, indicating that this compound has long-term duration effects on cognitive functions (Figs. 8 and 9).

It is also of great importance to note that AF64A (3 nmole/2ul/side) induced marked memory impairments in the Morris Swimming Maze test (Morris, loc. cit.), which AF102B (1 mg./kg., ip) counteracted, whereas physostigmine (0.1 mg./kg., ip) had a negative effect. Interestingly, the beneficial effects of AF102B in this test were on spatial memory dysfunctions induced by AF64A. In this regard, it is noteworthy that a main memory dysfunction in SDAT patients is impairment of spatial memory.

Experimental

Bilateral icv injections of AF64A produced cognitive impairments in rats in passive avoidance-step through and single-alternation tasks. Details are given below of studies on (a) the effect of AF64A on another behavioral paradigm, namely the Morris water maze; and (b) the possibility of reversal of the AF64A-produced effects by physostigmine as a reference compound, and by AF102B.

AF64A Preparation

AF64A was freshly prepared each day in 10 mM concentration and then diluted in artificial cerebrospinal fluid (CSF) to the proper concentration for injection. The composition of the artificial CSF pH 7.1-7.3 solution was:

	mM
NaCl	147
KCl	2.9
$MgCl_2 .6H_2O$	1.6
Dextrose	2.2
$CaCl_2 .2H_2O$	1.7

Step-through Passive Avoidance: Experiment 1

The effect of physostigmine on the performance and 24 hours retention of an inhibitory learning (passive avoidance-step through) task, in AF64A and CSF injected rats was investigated, using a post-training drug treatment paradigm.

Male Sprague-Dawley(raised by Charles River) rats, 90-110 days old, weighing 230-360 g., were all allowed free access to food and water. Prior to surgical procedure, rats were anesthetized with Equithesin (0.3 ml./ 100 g. ip). Bilateral injections were made by stereotaxic application of AF64A or

vehicle into the lateral cerebroventricles (icv) (AP - 0.8 mm. from bregma, L-1.5 mm. from bregma and - 4.7 mm. from skull surface). 23 rats were infused with 3 mole of AF64A in a volume of 2µl*, into each lateral ventricle (group 1), and 20 control rats were infused similarly with the same volume of CSF (group 2). Altogether 43 rats were operated. The infusion was made by a 28-ga. injection cannula. the rate of injection was kept constant at 0.25 µl/ min. The injection cannula was left in place for 4 mins. after injection to allow diffusion of the solutions into the ventricles.

27-28 days after injection**, each group of rats was post-operatively subdivided randomly into 2 groups of 10 rats each; subgroup 1 was assigned to the physostigmine treatment and subgroup 2 was treated with saline. Each rat was individually placed in a small lighted front compartment of a two-compartment box. After a 60 sec. familiarization/adaptation period, the door separating the two compartments was opened and a clock activated. The rat's latency to enter the large dark compartment of the box (to step through) was measured. Immediately following entry into the dark compartment, the rat was subjected to an inescapable scrambled foot shock applied to the grid floor (0.6mA - for 3 secs.). 60 secs after the termination of the shock, at the end of the training procedure, the rat was removed from the dark compartment and physostigmine dissolved in saline (0.06 mg./kg.) or saline placebo were administered ip. Rats were then returned to their home cage. Retention of the passive avoidance task was measured 24 hrs. after training by again placing the rat in the

lighted front compartment and after a 60 sec. adaptation period, measuring the latency to enter the dark compartment. The test session ended when a rat entered the dark compartment, or after 600 secs. had elapsed. Animals that failed to step through within 600 secs. were removed from the apparatus and a 600 secs. latency was recorded for them.

*This dose was found effective in impairing the passive avoidance step-through performance of rats.
**The 27-day interval was found to be effective in impairing the passive avoidance step-through performance in 3 nmole/2ul AF64A-injected rats.

Results: Mortality and Body Weight.

Immediately following surgery the AF64A-injected animals appeared unresponsive to environmental stimuli. A few of the animals (2 out of 20 in the AF64A-injected group and 6 out of 20 in the CSF-injected group) showed a loss of body weight (2% and 12.5%, respectively), which was observed 7 days following dosing. Within 48 hours of dosing there was a cumulative mortality of 13% of the AF64A-injected group.

Results: Passive Avoidance Test.

The initial latency measures and the retention-test latency measures were analyzed by a two-way ANOVA, Injection (AF64A/CSF) vs. Treatment (physostigmine/saline). Table (i) presents the mean - S.E.M. of the initial latency measures, while Table (ii) presents the mean - S.E.M. of the retention-test latency measures.

Table (i)

Initial-test latency measures (secs.)

Injection Pre-Treatment	AF64A 3 nmole/2ul	CSF 2µl
Physostigmine (0.06 mg./kg.)	25.50-5.68	29.70-7.93
Saline	33.80-9.66	16.10-3.73

Table (ii)

Retention-test latency measures (secs.)

Injection Treatment	AF64A 3 nmole/2ul	CSF 2µl
Physostigmine (0.06 mg./kg.)	458.90-63.33	576.10-22.69
Saline	247.90-52.26	556.60-28.44

No significant differences were found during the training trial between any of the groups tested (see Fig. 6): $F(1,36) = 0.81$, $p>0.05$.

The step-through latency of the AF64A-injected group was significantly shorter, $F(1,36) = 20.18$, $p<0.01$ during the 24hr. retention test than the CSF-injected group's latency. In addition, the step-through latency of the physostigmine-treated group was significantly longer, $F(1,36) = 5.91$, $p<0.05$, during the 24hr. retention-test than the saline-treated group's latency. Retention of a step-through passive avoidance response of the AF64A-injected group was significantly improved by physostigmine

administration while the retention of the CSF-injected group was not affected by this treatment (see Fig. 6), $F(1,36) = 4.08$, $p = 0.05$. Shiffe's contrasts of the step through latency measures during the retention test revealed a significant difference between the saline-treated AF64A and CSF-injected groups ($p<0.05$). The three other contrasts were not significant although the latency of the saline-treated AF64A-injected group appears to be shorter than the latency of the physostigmine-treated AF64A-injected group.

Step-through Passive Avoidance: Experiment 2

The effects of AF102B on the performance and 24 hours retention of an inhibitory learning (passive avoidance-step-through) task, in AF64A and CSF injected rats were investigated, using a post-treatment drug paradigm.

The surgery procedure was identical to that of Experiment 1 except that (1) the weight of the operated rats was 250-325 g.; (2) 20 rats were infused with 3 nmole of AF64A in a volume of 2µl, into each ventricle (group 1), and 20 control rats were infused with a volume of 2µl of CSF into each lateral ventricle (group 2). Altogether 40 rats were operated.

The behavioral testing procedure comprised 4 phases. Pretest: 28 male Sprague-Dawley naive rats, 90-110 days old, weighing 270-310 g. were randomly divided into 4 groups of 7 rats each; 3 groups were assigned to AF102B dissolved in saline treatments (0.1, 1 or 5 mg./kg.) and the fourth group was treated with saline. The training and test-retention procedure was

identical to that in Experiment 1 except that at the end of the training procedure different doses of AF102B or saline placebo were administered ip.

Phase 1: 27-28 days after injection each group of rats was postoperatively subdivided randomly into 2 groups of 10 rats each; subgroup 1 was assigned to the AF102B treatment and subgroup 2 was treated with saline. The training and the test-retention procedure was identical to that of Experiment 1 except that at the end of the training procedure, the rat was removed from the dark compartment and doses of AF102B dissolved in saline (1 mg./kg.) or saline were administered ip.

Phase 2: 6 days after the retention test, rats were subjected to 6 daily sessions of extinction procedure. The rat was placed in the lighted front compartment and the latency to enter the dark compartment was measured. This procedure was identical to the retention test's procedure in Phase 1.

Phase 3: After the extinction procedure, rats were subjected to a latent extinction procedure. The rat was placed in the lighted front compartment and after a 60 sec. adaptation period, was pushed into the dark compartment and kept there for 60 secs. This procedure was followed once a day for three days. After the latent-extinction procedure, rats were subjected to an "extinction + latent extinction" procedure. The rat was placed in the lighted front compartment and after a 60 sec. adaptation period, the latency to enter the dark compartment was measured. The test session ended when a rat entered the dark compartment. Animals that failed to step through within 600 secs. were pushed into the dark compartment and a 600 secs. latency was recorded

for them. This procedure was followed once a day for 4 days. At the end of the 4th session, immediately following entry into the dark compartment, the rat was subjected to a training and retention-test procedure identical with that in Phase 1 except that the two sub-groups treated in Phase 1 with AF102B were now treated with saline placebo and vice-versa. (It should be noted that this treatment took place 2 months after the AF64A/CSF injection).

Results: Mortality and Body Weight.

Immediately following surgery the AF64A-injected animals appeared unresponsive to environmental stimuli. A few of the animals (2 out of 20 in the AF64A-injected group) showed a 2% loss of body weight which was observed 7 days following dosing. Within 48 hours of dosing no mortality occurred in any of the groups.

Results: Passive Avoidance Test.

Pretest.

The initial latency measures and the retention-test latency measures were analyzed by a one-way ANOVA. Table (iii) presents the mean-S.E.M. of the initial latency measures, while Table (iv) presents the mean-S.E.M. of the retention-test latency measures.

Table (iii)
Initial latency measures (secs.)

AF102B 0.1 mg./kg.	AF102B 1 mg./kg.	AF102B 5 mg./kg.	Saline
16.71 - 4.77	11.86 - 1.23	19.71 - 5.39	20.00 - 5.64

Table (iv)
Retention-test latency measures (secs.)

AF102B 0.1 mg./kg.	AF102B 1 mg./kg.	AF102B 5 mg./kg.	Saline
330.57 - 75.55	348.14 - 81.66	551.71 - 32.92	513.00 - 56.39

No significant differences were found during the training trial between any of the groups tested, $F(3,24) = 0.57$; $p>0.05$. In addition, no significant differences were found during the 24 hr. retention-test between the different doses of AF102B, $F(3,24) = 2.61$; $p>0.05$, although the retention latency of the 0.1 mg./kg. and the 1 mg./kg/ groups tends to be shorter than the latency of the 5 mg./kg. and the saline groups.

Phase 1.

The initial latency measures and the retention-test latency measures were analyzed by a two-way ANOVA, Injection (AF64A/CSF) vs. Treatment (AF102B/Saline). Table (v) presents the mean - S.E.M. of the initial latency measures, while Table (vi) presents the mean - S.E.M. of the retention-test latency measures.

Table (v)

Initial-test latency measures (secs.)

Injection Treatment	AF64A 3 nmole/2µl	CSF 2µl
AF102B (1 mg./kg.)	18.40 - 3.74	15.30 - 2.83
Saline	22.66 - 4.43	15.20 - 2.10

Table (vi)

Retention-test latency measures (secs.)

Injection Treatment	AF64A 3 nmole/2µl	CSF 2µl
AF102B (1 mg./kg.)	514.10 - 38.21	447.60 - 58.09
Saline	182.44 - 29.90	574.30 - 16.39

No significant differences were found, during the training trial, between any of the groups tested (see Fig. 6). : $F(1, 35) = 2.16$, $p>0.05$ for the injection variable (AF64A/CSF) main effect. For the other main effect (the two groups to be treated with AF102B or saline) : $F(1,35) = 0.29$; $p>0.05$.

The step-through latency of the AF64A-injected group was significantly shorter, $F(1,35) = 13.89$; $p<0.01$, during the 24 hr. retention test than the CSF-injected group's latency. In addition, the step-through latency of the AF102B-treated group was significantly longer, $F(1,35) = 4.98$; $p<0.05$, during the 24 hr. retention test than the saline-treated group's latency.

Retention of a step-through passive avoidance response of the AF64A-injected group was significantly improved by AF102B administration, while that of the CSF-injected group was significantly impaired (see Fig. 7), $F(1,35) = 31.18$, $p<0.01$. Shiffe's contrasts of the step through latency measures during the retention test revealed that all the between-groups differences were significant ($0.01 < p < 0.05$) except the AF64A + AF102B vs. CSF + AF102B difference.

Phase 2.

The retention-test latency measures during the extinction period

was analyzed by a three-way ANOVA (6x2x2) with one repeated variable (Trials) and two non-repeated variables (Injection - AF64A/CSF and Treatment - AF102B/CSF). Fig. 8 shows that the step-through latency of the AF64A-injected group was significantly shorter, $F(1,36) = 16.83$; $p<0.01$, than the CSF-injected group's latency. In addition, the step-through latency of the AF102B-treated group was significantly longer, $F(1,36) = 31.45$, $p<0.01$, than the saline-treated group's latency. Retention of a step through-passive avoidance response of both the AF64A and the CSF-injected groups, during extinction, was significantly inproved by AF102B administration. $F(1,36) = 15.80$, $p<0.01$. Shiffe's contrasts of the step-through latency measures during the extinction period revealed that all the between-groups differences were significant ($p<0.01$) except the AF64A + AF102B vs. CSF + AF102B difference.

The trials variable main effect during extinction was significant, $F(5,180) = 2.68$, $p<0.05$. Contrasts analysis showed that the retention latency of the second extinction trial was significantly longer than the latency of the 6th trial ($p<0.05$). There were no other significant differences.

The interaction between treatment and trials was significant, $F(5,180) = 3.30$, $p<0.05$. Significant simple main effects contrasts were found between the AF102B-treated groups and the saline-treated groups during all the extinction trials except the first trial. In addition, the retention latency extinction curve was significantly different from that of the saline-treated groups ($p<0.05$). The retention latency of the AF102B-treated

groups increased from the first to the fourth trial and then decreased. The retention latency of the saline-treated groups decreased from the first trial to the sixth trial.

Phase 3.

A. The retention-test latency measures during the "extinction + latent extinction" period was analyzed by a three-way ANOVA (4x2x2) with one repeated variable (Trials) and two non-repeated variables (Injection - AF64A/CSF and Treatment AF102B/CSF). No significant differences were found during "extinction + latent extinction" period between any of the conditions tested, in the retention-test latency measures, although the retention latency of the saline-treated AF64A-injected group appears to be shorter than the retention latency of the three other groups (see Fig. 9).

B. The retention-test latency measures of the second administration of AF102B/Saline were analyzed by a two-way ANOVA, Injection (AF64A/CSF) vs. Treatment (AF102B/Saline). Table (vii) presents the mean - S.E.M. of the retention-test latency measures.

Table (vii)

Retention-test latency measures (secs.)
of second administration of AF102B/Saline

Injection Treatment	AF64A 3 nmole/2µl	CSF 2µl
AF102B (1 mg./kg.) (previosly Saline)	580.40 - 18.61	600 - 0
Saline (previously AF102B)	600 - 0	600 - 0

No significant differences were found following the second AF102B/Saline administration between any of the groups tested ($p>0.05$) (see Fig. 10). The retention latency of the Saline-treated AF64A-injected group, previously treated with AF102B, remained in the same level, while the retention latency of the AF102B-treated AF64A-injected group, previosly treated with saline, increased and reached the level of the CSF-injected groups.

Morris Swimming Maze

Cholinergic deficiency, as well as treatments with anticholinergic drugs, were shown to impair memory and learning processes associated with spatial orientation (Sutherland et al, J. Comp. Physiol. Psychol. 96: 563-73, 1982). In this respect, the water-maze (Morris, Learning and Motivation, 12: 239-61, 1978) seemed to be a suitable behavioral paradigm for the detection of cognitive impairments induced by AF64A in rats, and of their reversal by cholinergic drugs. Since physostigmine is currently one of the few cholinergic drugs used in Allzheimer's patients, it was selected for preliminary testing of its effects on AF64A treated rats.

38 male Sprague Dawley rats (raised by Charles River) 5-6 months old with an average weight of 500 g. were used. The rats were housed in groups of 5 and were given free access to food and water. Testing was carried out in a round white metal tub 1.4 m. diameter and 0.4 m. deep. The tub was filled with

water, made opaque by milk powder to the level of 18 cm. A platform of 12 cm. diameter and at a height of 16 cm. was placed in the tub 2 cm. below water level. The platform was covered with gauze to prevent the animal from slipping back into the water after reaching the platform.

Prior to testing, the rat was placed on the platform for 120 secs., placed gently into the water, facing the wall of the pool, at one of four starting locations (north, south, east or west) around the pool's perimeter. Within each block of four trials, each rat was started at each of the four starting locations, with the sequence of starting locations randomly selected. Testing was carried out on 2 consecutive days with each rat submitted daily to eight trials. During trials 1-12 the platform was located in the center of the south-east quadrant and during trials 13-16 the platform was transferred to the center of the north-west quadrant. If in a particular trial the rat found the platform, it was permitted to remain on it for 60 secs. before starting the next trial. If a rat failed to find the platform, the trial was terminated after a cut-off time of 120 secs., and subsequently was put on it for 60 secs. before starting the next trial. The latency to find the platform was measured.

For drug testing, rats were injected with AF64A or CSF (3nmole/2µl/side) as described in Experiment 2, above. The rats were subjected first to a step-through learning procedure (see Experiment 1, above) and were 3-3.5 months after injection when tested in the water maze. The rats were divided into 4 groups; 18 CSF-injected rats were treated either with physostigmine (0.1

mg./kg. ip)(10 rats) or with saline (8 rats). 20 AF64A-injected rats were divided similarly. The rats were injected with physostigmine immediately before testing on each day.

Results.

(a) The escape latency was analyzed by a 3-way ANOVA (4x2x2) with one repeated variable (trials) and two non-repeated variables (Injection - AF64A/CSF and Treatment - physostigmine/saline). Table (viii) presents the escape-latency of the groups tested. Fig 11 depicts that AF64A injected rats showed an increase in escape latency (in seconds) relative to CSF treated rats. This effect is highly significant, $F(1,34) = 14.88$, $p<0.001$. The initial escape-latency for all four groups was similar. However, the escape-latency of the CSF-treated rats decreased faster than the escape-latency of the AF64A rats. Physostigmine apparently impaired the performance of both AF64A and CSF-injected groups, compared with non-treated AF64A and CSF-injected groups but this result was not statistically significant. The trials effect was statistically significant, $F(15,510) = 5.9$, $p<0.001$. In addition, a significant interaction between trials and physostigmine was found, $F(15,510) = 4.3$, $p<0.001$; physostigmine inhibited the decrease of the escape-latency curve, regardless of the injected group.

(b) Table (viii) shows that there was a large variation within the groups, a fact which may be the reason for the non-significant differences between the groups treated with physostigmine and those that received no treatment.

Table (viii)

Escape-latency of AF64A and CSF-injected rats under physostigmine or saline treatment

Trial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
$\bar{x}$ AF64A	75.1	36.5	69.5	65.3	56.8	54.4	60	59.7	46.6	48.1	29	21.9	58.3	29.8	26.9	14.5
SEM r = 10	14.9	12.4	14.5	48.6	16.5	15.8	16.8	17.1	12.3	13.8	7.9	8.8	13.9	10.7	8.1	2.8
$\bar{x}$ CSF	85.5	56.8	24.6	25.1	22.7	18.1	7.25	25.7	29.2	18.7	23	8.5	31.6	18.5	14.6	8.8
SEM r = 8	15.5	14.7	8.59	10.4	5.2	5.0	1.6	13.8	14.1	7.2	14	1.9	13.8	5.1	3.5	2.27
$\bar{x}$ CSF+Physostigmine	100.2	62.7	44.3	25.7	35.7	24.2	34.1	30	25.5	20.1	7.9	17.2	47	34.3	31.8	10.9
SEM r = 10	10.7	16.6	16.2	10.2	14.7	11.4	14.5	11.7	10.5	8.8	2.3	6.5	10.5	13	14.8	2.1
$\bar{x}$ AF64A+Physostigmine	98.5	99.5	81	71.9	87.3	78.1	70.2	90.7	66.1	46.8	41.1	45	67.8	32.9	49.9	55.5
SEM r = 10	10.2	12.5	16.1	16.3	16.6	12.9	14.3	13.3	15.4	14.2	13.9	13.8	17.6	11.4	14.1	13.7

When similar Morris Swimming Maze experiments were carried out on AF102B (1 mg./kg., ip), it was found that this compound definitely improved the memory deficits induced by AF64A, whereas physostigmine (0.1 mg./kg., ip) had a negative effect. The beneficial effects of AF102B in this test were on spatial memory dysfunctions induced by AF64A. It is noteworthy that in this respect, a main memory dysfunction in SDAT patients is impairment of spatial memory.

CLAIMS

1. Quinuclidine derivatives having the general formula (I)

and geometrical isomers, enantiomers, diastereoisomers, racemates and/or acid addition salts thereof, wherein Z represents the group $>CR^1R^2$ or two hydrogen atoms; and R^1 and R^2, which may be identical or different, are each alkyl, cyclopentyl, cyclohexyl, aryl or diarylmethylol, or alkyl which is substituted by one or more aryl groups, or one of R^1 and R^2 may be hydrogen.

2. Quinuclidine derivatives according to claim 1, wherein Z represents the group $>CR^1R^2$, one of R^1 and R^2 is hydrogen, and the other of R^1 and R^2 is alkyl, cyclopentyl, cyclohexyl, aryl or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

3. Quinuclidine derivatives according to claim 1, wherein

Z represents the group $>CR^1R^2$, one of R^1 and R^2 is alkyl, cyclopentyl, cyclohexyl, and the other of R^1 and R^2 is alkyl, cyclopentyl, cyclohexyl, aryl or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

4. Quinuclidine derivatives according to claim 1, wherein Z represents the group $>CR^1R^2$, one of R^1 and R^2 is aryl, and the other of R^1 and R^2 is aryl or diarylmethylol, or alkyl which is substituted by one or more aryl groups.

5. A quinuclidine derivative as defined in claim 2, wherein one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is methyl.

6. A quinuclidine derivative as defined in claim 2, wherein one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is phenyl.

7. A quinuclidine derivative as defined in claim 2, wherein one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is diphenylmethyl.

8. A quinuclidine derivative as defined in claim 2, wherein one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is selected from the group consisting of ethyl, propyl, 1-pyrenepropyl and diphenylmethylol.

9. A quinuclidine derivative as defined in claim 3, wherein one of R^1 and R^2 is methyl and the other of R^1 and R^2 is phenyl.

10. A quinuclidine derivative as defined in claim 3, wherein one of R^1 and R^2 is phenyl and the other of R^1 and R^2 is selected from the group consisting of ethyl and cyclohexyl.

11. A quinuclidine derivative as defined in claim 4, wherein R^1 and R^2 are each phenyl.

12. A quinuclidine derivative as defined in claim 1, wherein Z represents two hydrogen atoms.

13. The geometrical isomer of the compound defined in claim 5, the hydrochloric acid salt of which has the relatively lower melting-point.

14. The geometrical isomer of the compound defined in claim 5, the hydrochloric acid salt of which has the relatively higher melting-point.

15. The hydrochloric acid salt of the compound defined in claim 5.

16. The relatively lower melting-point geometrical isomer of the compound defined in claim 15.

17. The relatively higher melting-point geometrical isomer of the compound defined in claim 15.

18. A process for preparing quinuclidine derivatives according to claim 1, and wherein Z represents the group $>CR^1R^2$, which comprises reacting the quinuclidine derivative defined in claim 12 with a ketone of formula R^1-CO-R^2, and isolating the desired product from the reaction mixture.

19. A process according to claim 18, which is carried out in the presence of an acid catalyst.

20. A process according to claim 19, wherein the catalyst is a Lewis acid.

21. A process according to claim 20, wherein the Lewis acid is boron trifluoride.

22. A process for preparing quinuclidine derivatives according to claim 1, and wherein Z represents the group $>CR^1R^2$, which comprises reacting the quinuclidine derivative defined in claim 12 with a ketone of formula R^1-CO-R^2, in an atmosphere of nitrogen, at a temperature in the range of about 20 to about 30 °C, in the presence of boron trifluoride etherate as catalyst and in dichloromethane as the solvent medium, and isolating the desired product from the reaction mixture.

23. A process according to claim 22, wherein the reaction is effected at a temperature of about 25 °C.

24. A process according to either claim 22 or claim 23, wherein the reaction ingredients are first mixed in an atmosphere of nitrogen at a temperature between about -10 and +20 °C, and the mixture thus obtained is permitted to rise to the reaction temperature.

25. A process according to claim 24, wherein the mixing temperature is about 0 °C.

26. A process according to either claim 18 or claim 22,

wherein following isolation the desired product is separated into its geometrical isomers.

27. A process according to claim 26, wherein the separation is effected by fractional crystallization.

28. A process according to either claim 18 or claim 22, wherein the product is isolated as the free base and thereafter converted to its acid addition salt.

29. A process according to either claim 18 or claim 22, wherein the product is isolated in the form of an acid addition salt and thereafter converted to the free base.

30. A process according to any of claims 18 to 21, wherein the reaction between the compound defined in claim 12 and the said ketone is carried out in the presence of an inert organic solvent.

31. A process according to claim 30, wherein the inert organic solvent is dichloromethane.

32. A process for preparing the compound defined in claim 12, wherein the epoxide of 3-methylenequinuclidine is reacted with hydrogen sulfide.

33. A process according to claim 32, wherein the reaction is carried out in presence of a base.

34. A process according to claim 33 wherein the base is sodium hydroxide.

35. A process according to any of claims 32 to 34, which is carried out in an aqueous medium.

36. A process according to any of claims 32 to 35, wherein the said epoxide is prepared by reacting quinuclidin-3-one with dimethylsulfoxonium methylide.

37. A process according to any of claims 18 to 31, wherein the said compound which is defined in claim 12 is prepared by reacting the epoxide of 3-methylenequinuclidine with hydrogen sulfide.

38. A process according to claim 37, wherein the reaction of epoxide with hydrogen sulfide is carried out in presence of a base.

39. A process according to claim 38 wherein the base is sodium hydroxide.

40. A process according to any of claims 37 to 39, wherein the reaction of epoxide with hydrogen sulfide is carried out in an aqueous medium.

41. A process according to any of claims 37 to 40, wherein the said epoxide is prepared by reacting quinuclidin-3-one with dimethylsulfoxonium methylide.

42. A pharmaceutical composition which comprises a quinuclidine derivative of formula (I) as defined in claim 1, and wherein Z represents the group $>CR^1R^2$, or a pharmaceutically compatible acid addition salt thereof, together with an inert carrier or diluent.

43. A pharmaceutical composition according to claim 42, which is in a form suitable for oral, rectal or parenteral administration, or for administration by insufflation.

44. A pharmaceutical composition according to claim 42, which is in a form suitable for transdermal administration.

45. A pharmaceutical composition according to any of claims 42 to 44, which is in unit dosage form.

46. A pharmaceutical composition for transdermal administration, which comprises a quinuclidine derivative of formula (I) as defined in claim 1, wherein Z represents the group $>CR^1R^2$, or a pharmaceutically compatible acid addition salt thereof, as well as a low molecular weight fatty acid.

47. A pharmaceutical composition according to any of claims 42 to 46, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is phenyl, and the other of R^1 and R^2 is selected from the group consisting of ethyl, cyclohexyl and phenyl.

48. A pharmaceutical composition according to any of claims 42 to 46, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is hydrogen, and the other of R^1 and R^2 is either methyl or ethyl.

49. A pharmaceutical composition according to any of claims 42 to 46, wherein the quinuclidine derivative of formula (I) is that defined in claim 13.

50. A pharmaceutical composition according to claim 49, which contains additionally one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, aniracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin.

51. A pharmaceutical composition according to any of claims 42 to 46, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl, diarylmethylol, and alkyl substituted by aryl, and the other of R^1 and R^2 is as previously defined.

52. A pharmaceutical composition according to claim 51, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is methyl and the other of R^1 and R^2 is phenyl.

53. A pharmaceutical composition according to claim 51, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is hydrogen and the other of R^1 and R^2 is diphenylmethyl.

54. A pharmaceutical composition according to claim 51, wherein the quinuclidine derivative of formula (I) is that in which one of R^1 and R^2 is hydrogen, and the other of R^1 and R^2 is selected from the group consisting of propyl, phenyl, 1-pyrenepropyl, and diphenylmethylol.

55. A quinuclidine derivative as defined in claim 1 wherein Z represents the group $>CR^1R^2$, or in any of claims 2 to 11 and 13 to 17, when prepared by the process of any of claims 18 to 31 and 37 to 41.

56. A quinuclidine derivative as defined in claim 12, when prepared by the process of any of claims 32 to 36.

57. A method for treating diseases of the central nervous system in mammals, which comprises administering to the mammal a quinuclidine derivative, or a pharmaceutically compatible acid addition salt thereof, as defined in claim 1 wherein Z represents the group $>CR^1R^2$, or as defined in any of claims 2 to 11, 13 to 17, and 55.

58. A method for treating diseases of the central nervous system in mammals, which comprises administering to the mammal a pharmaceutical composition as defined in any of claims 42 to 54.

59. A method for treating diseases of the central nervous system in mammals, which comprises transdermal administration to the mammal of a quinuclidine derivative, or a pharmaceutically compatible acid addition salt thereof, as defined in claim 1 wherein Z represents the group $>CR^1R^2$, or as defined in any of claims 2 to 11, 13 to 17, and 55.

60. A method for treating diseases due to a deficiency in the central cholinergic system in mammals, which comprises administering to the mammal a quinuclidine derivative as defined in any of claims 5 and 13 to 17.

61. A method for treating diseases due to a deficiency in the central cholinergic system in mammals, which comprises administering to the mammal a pharmaceutical composition containing a quinuclidine derivative as defined in any of claims 5 and 13 to 17, together with an inert carrier or diluent.

62. A method for treating diseases due to a deficiency in the central cholinergic system in mammals, which comprises transdermal administration to the mammal of a quinuclidine derivative as defined in any of claims 5 and 13 to 17.

63. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a quinuclidine derivative, or a pharmaceutically compatible acid addition salt thereof, as defined in claim 1 wherein Z represents the group $>CR^1R^2$, one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl, diarylmethylol, and alkyl substituted by aryl, and the other of R^1 and R^2 is as previously defined.

64. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a pharmaceutical composition containing a quinuclidine derivative, or a pharmaceutically compatible acid addition salt thereof, as defined in claim 1 wherein Z represents the group $>CR^1R^2$, one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl, diarylmethylol, and alkyl substituted by aryl,

and the other of R^1 and R^2 is as previously defined, together with an inert carrier or diluent.

65. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises transdermal administration to the mammal of a quinuclidine derivative, or a pharmaceutically compatible acid addition salt thereof, as defined in claim 1 wherein Z represents the group $>CR^1R^2$, one of R^1 and R^2 is selected from the group consisting of alkyl containing three or more carbon atoms, cyclopentyl, cyclohexyl, aryl, diarylmethylol, and alkyl substituted by aryl, and the other of R^1 and R^2 is as previously defined.

66. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a quinuclidine derivative as defined in claim 7, or a pharmaceutically compatible acid addition salt thereof.

67. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a pharmaceutical composition containing a quinuclidine derivative as defined in claim 7, or a pharmaceutically compatible acid addition salt thereof, together with an inert carrier or diluent.

68. A method for treating or diseases due to a deficiency in the central cholinergic hyperfunction in mammals, which comprises transdermal administration to the mammal of a quinuclidine derivative as defined in claim 7, or a pharmaceutically compatible acid addition salt thereof.

69. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a quinuclidine derivative as defined in claim 9, or a pharmaceutically compatible acid addition salt thereof.

70. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises administering to the mammal a pharmaceutical composition containing a quinuclidine derivative as defined in claim 9, or a pharmaceutically compatible acid addition salt thereof, together with an inert carrier or diluent.

71. A method for treating diseases due to cholinergic hyperfunction in mammals, which comprises transdermal administration to the mammal of a quinuclidine derivative as defined in claim 9, or a pharmaceutically compatible acid addition salt thereof.

72. A method for treating senile dementia of Alzheimer's type, which comprises administering to a patient a quinuclidine derivative as defined in claim 13, or a pharmaceutically compatible acid addition salt thereof, and optionally one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, aniracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin.

73. A method for treating senile dementia of Alzheimer's type, which comprises administering to a patient a pharmaceutical composition containing a quinuclidine derivative as defined in

claim 13, or a pharmaceutically compatible acid addition salt thereof, and optionally one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, choline, lecithin, piracetam, amiracetam, pramiracetam, oxiracetam, 4-aminopyridine, 3,4-diaminopyridine and somatostatin, together with an inert carrier or diluent.

74. A method for treating senile dementia of Alzheimer's type, which comprises transdermal administration to a patient of a quinuclidine derivative as defined in claim 13, or a pharmaceutically compatible acid addition salt thereof, and optionally one or more compounds selected from the group consisting of physostigmine, tetrahydroaminoacridine, 4-aminopyridine and 3,4-diaminopyridine.

75. A quinuclidine derivative as claimed in any of claims 1 to 17, 55 and 56, substantially as described hereinbefore.

76. A process for preparing quinuclidine derivatives as claimed in any of claims 18 to 41, substantially as described hereinbefore.

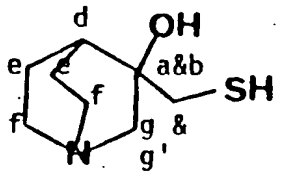
77. A pharmaceutical composition as claimed in any of claims 42 to 54, substantially as described hereinbefore.

78. A method as claimed in any of claims 57 to 74, substantially as described hereinbefore.

For the Applicants,

Sanford T. Colb,
Advocate and Patent Attorney
C: 03185

FIG. 1 NMR SPECTRUM OF 3- HYDROXY-3- MERCAPTO-METHYLQUINUCLIDINE



SOLVENT; $CDCl_3$
250 MHz- NMR

TMS

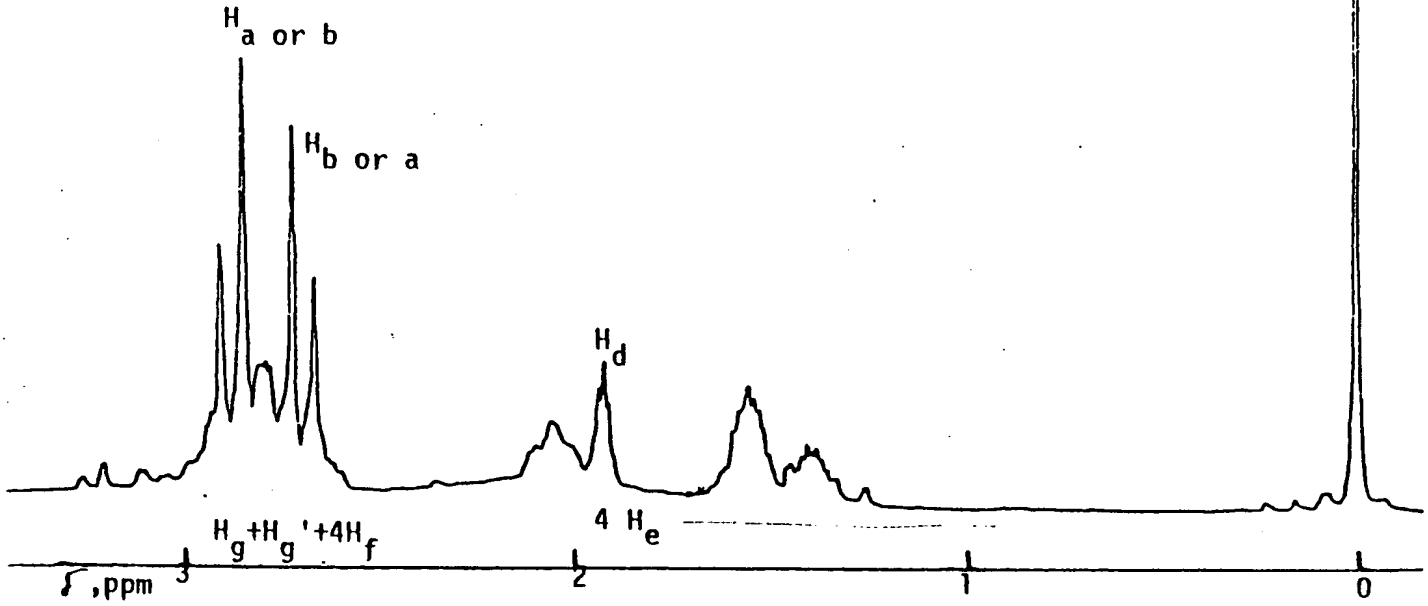
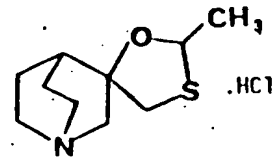
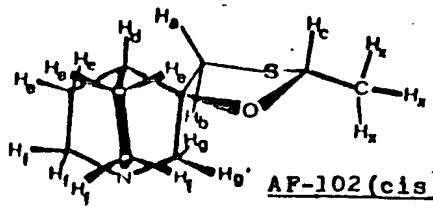


FIG.2 250 MHz- NMR of AF-102(cis: trans) the HCl salts in $CDCl_3$

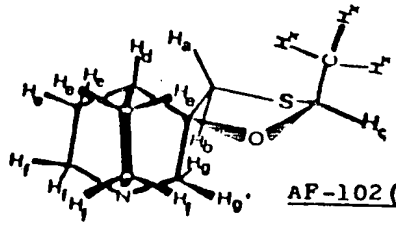


AF-102 (cis:trans)



AF-102(cis)

and



AF-102(trans)

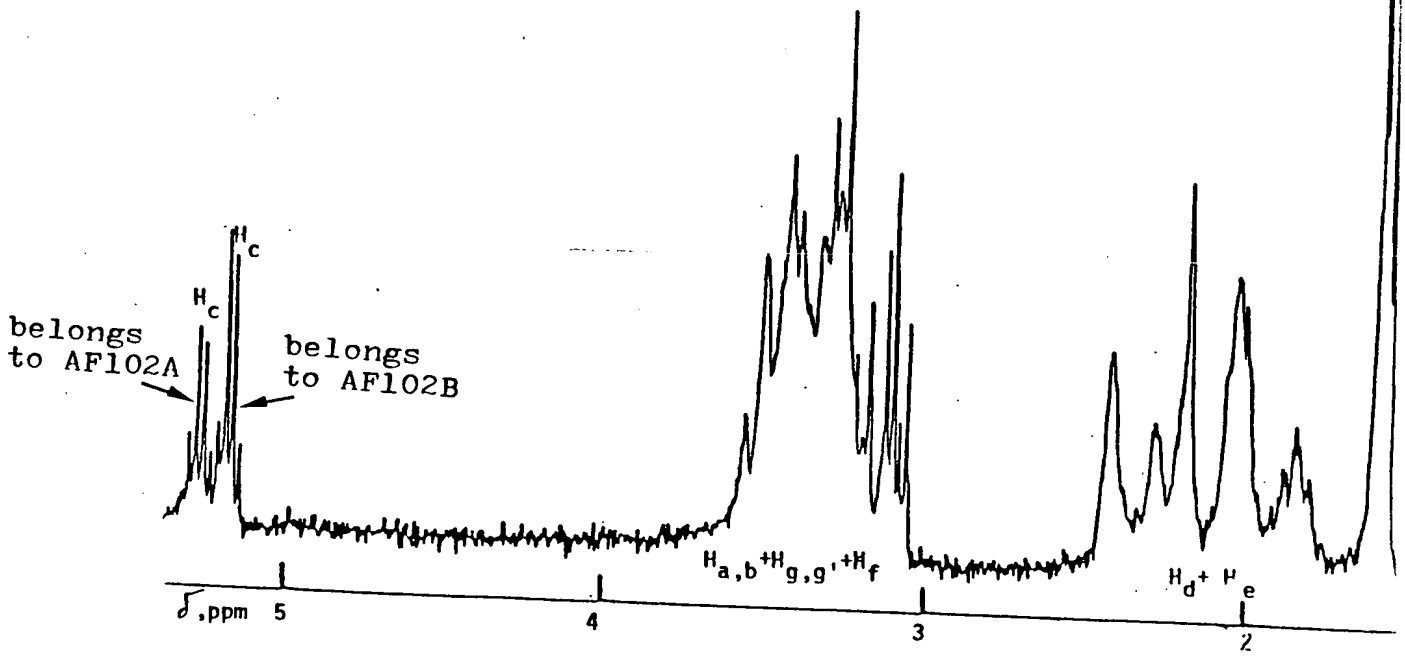
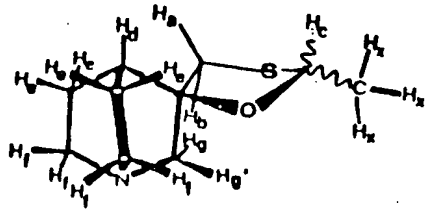
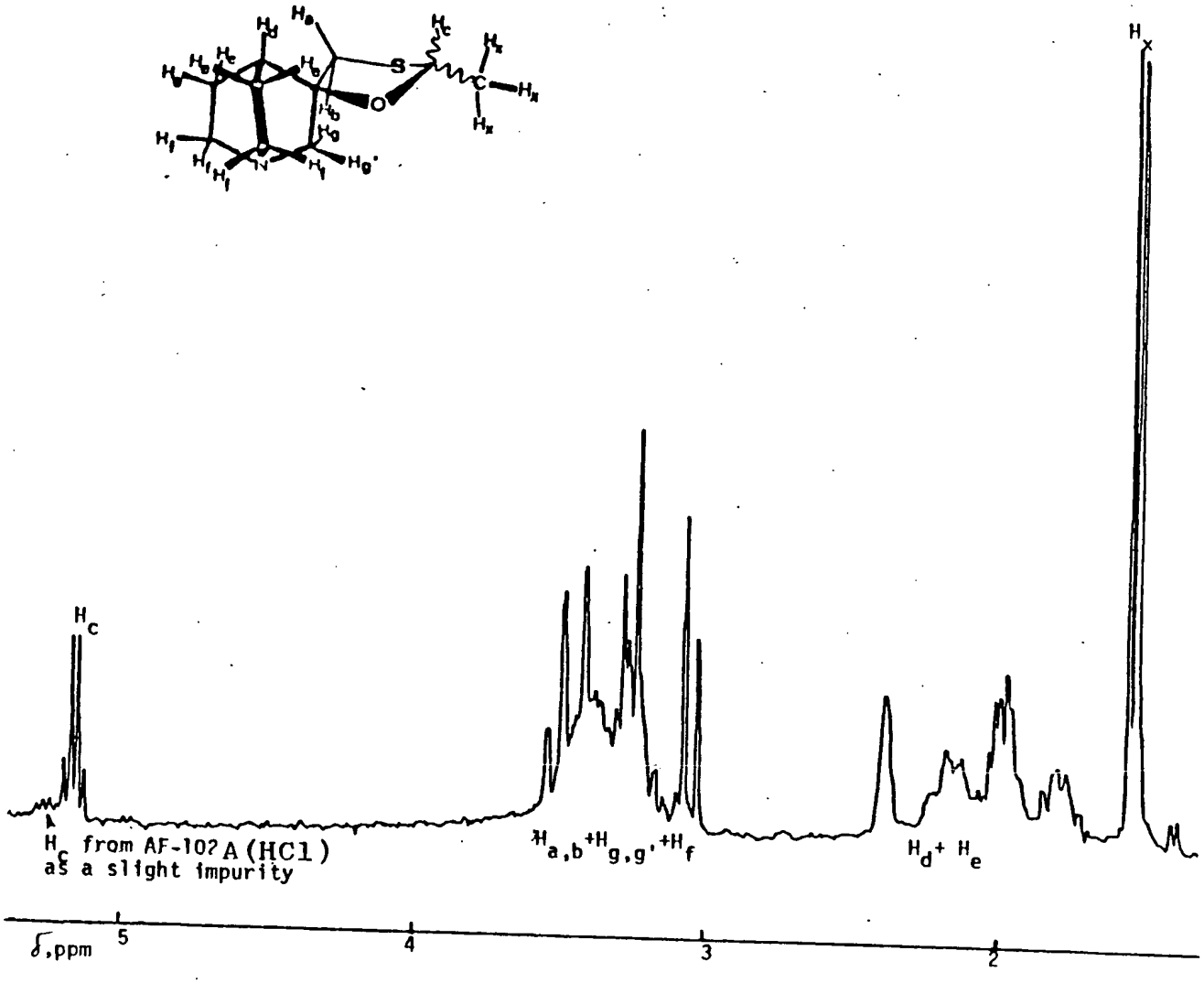


FIG. 3 250 MHz- NMR OF AF-102A (HCl)



SOLVENT: $CDCl_3$

FIG.4 250 MHz- NMR OF AF-102B (HCl) IN $CDCl_3$



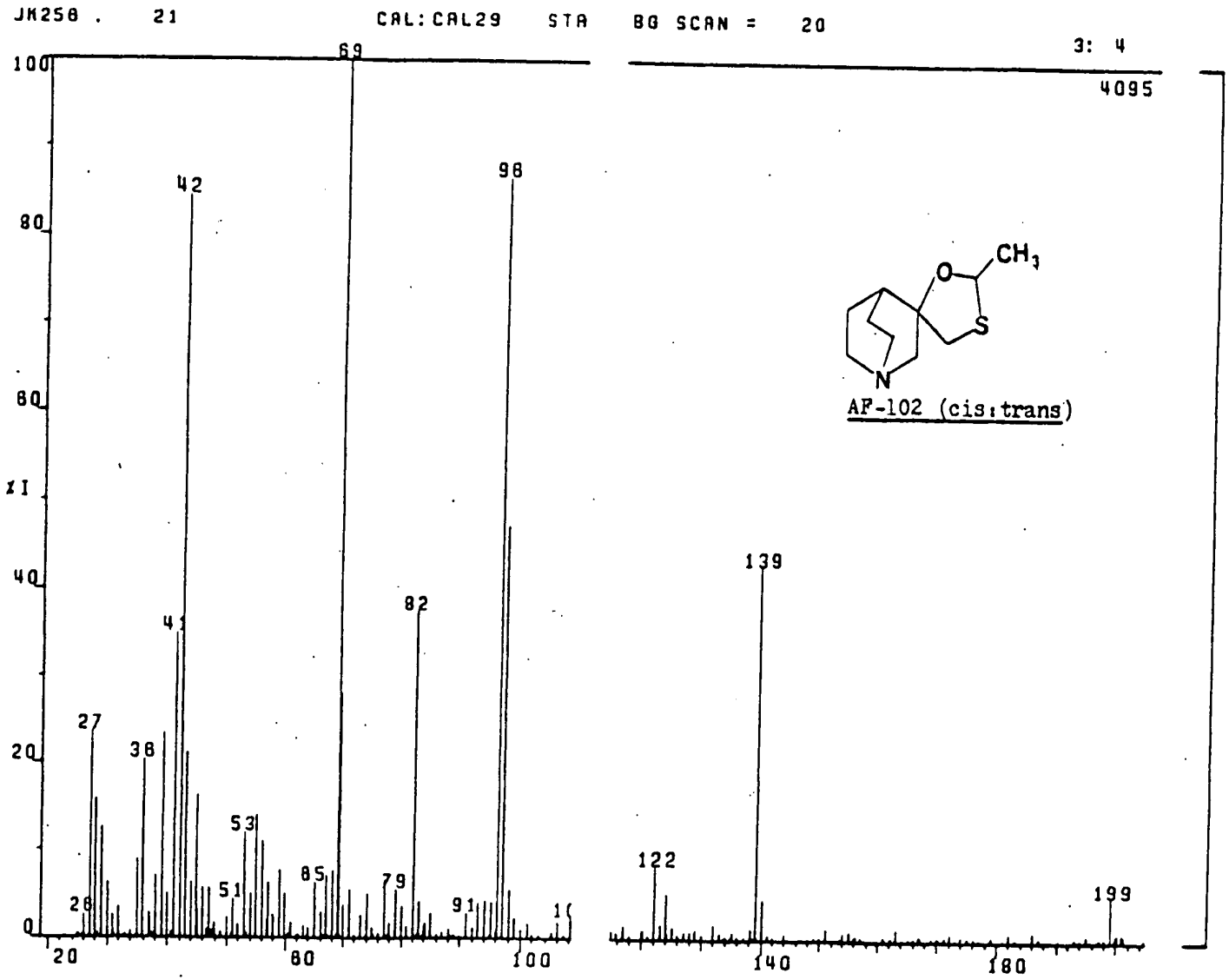


FIG. 5: MS OF AF102 (cis : trans)

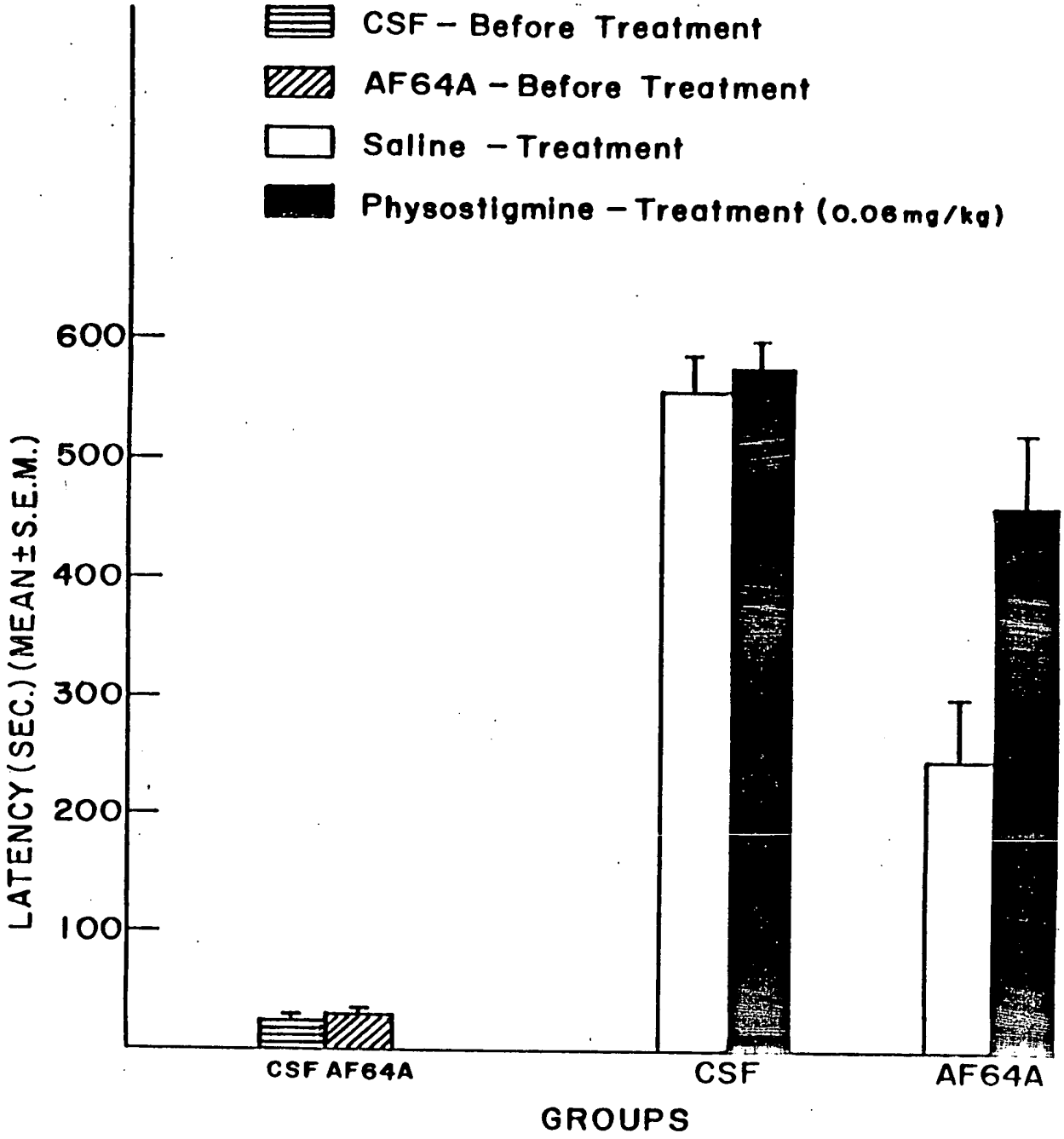


Fig. 6: Initial latency measures and retention-test latency measures (sec.) of the AF64A and CSF-injected groups, before and after physostigmine or saline administration.

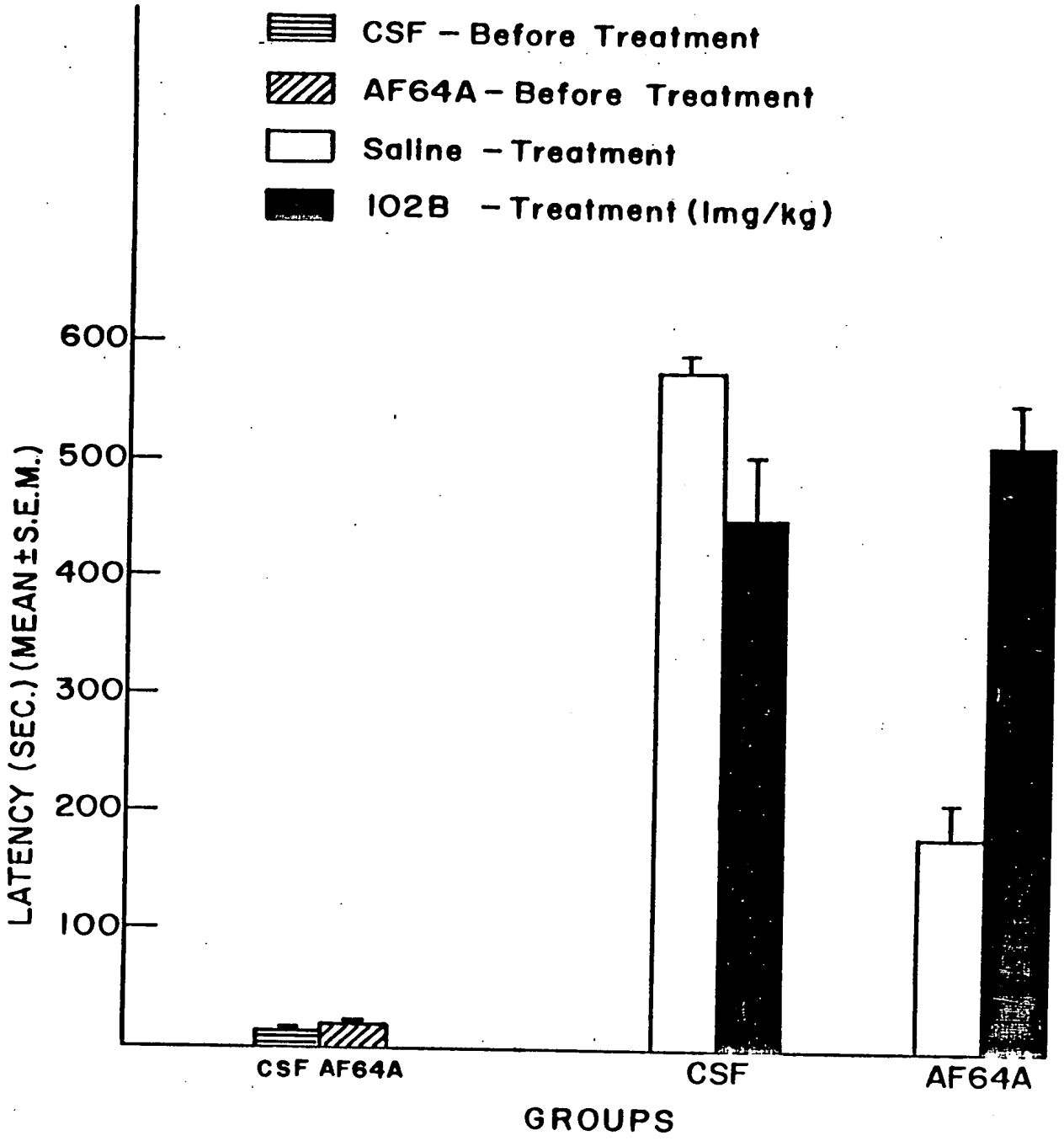


Fig. 7: Initial latency measures and retention-test latency measures (sec.) of the AF64A and CSF-injected groups, before and after AF102B or saline administration.

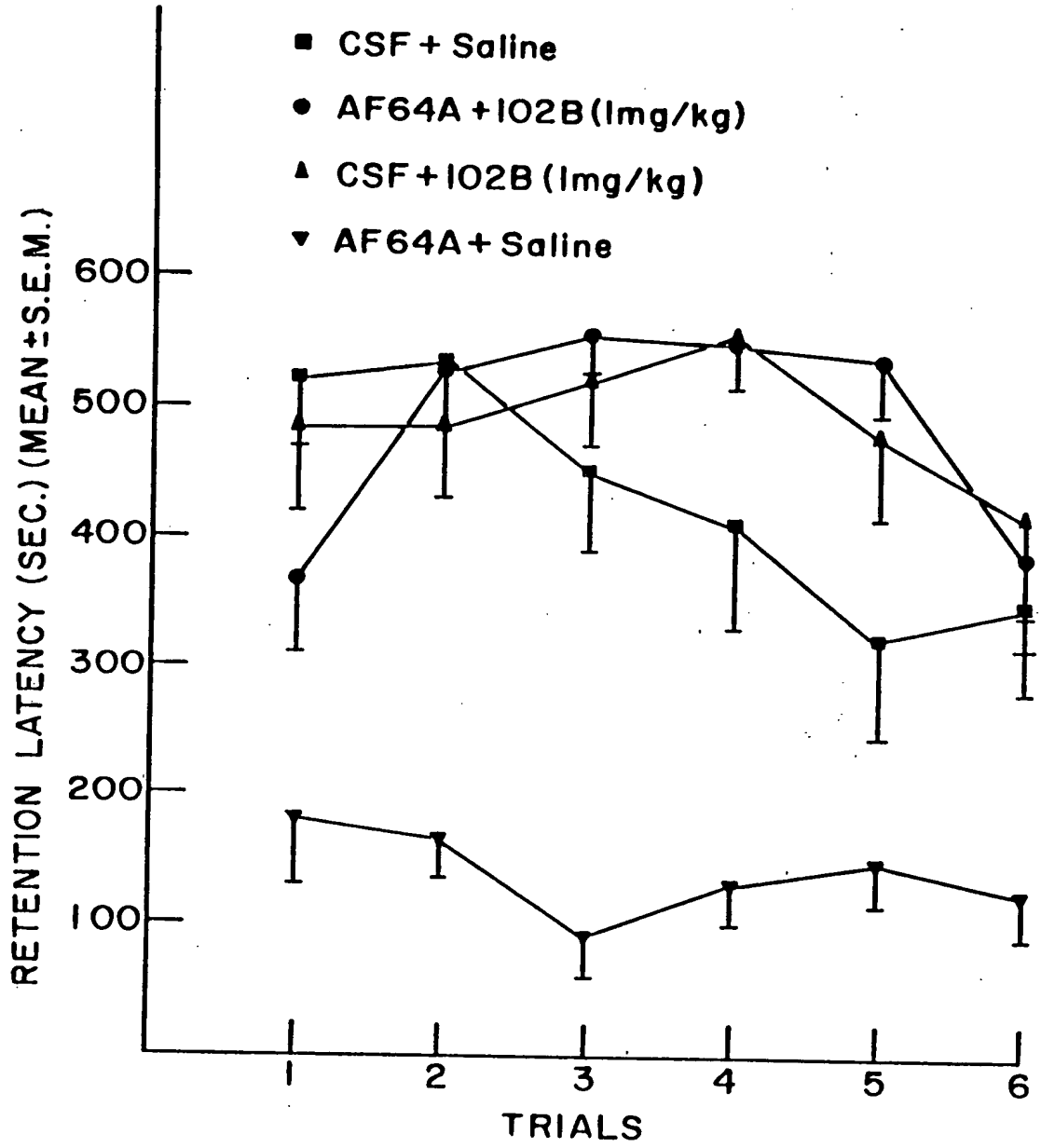


Fig. 8 : Retention-test latency measures during 6 trials of extinction.

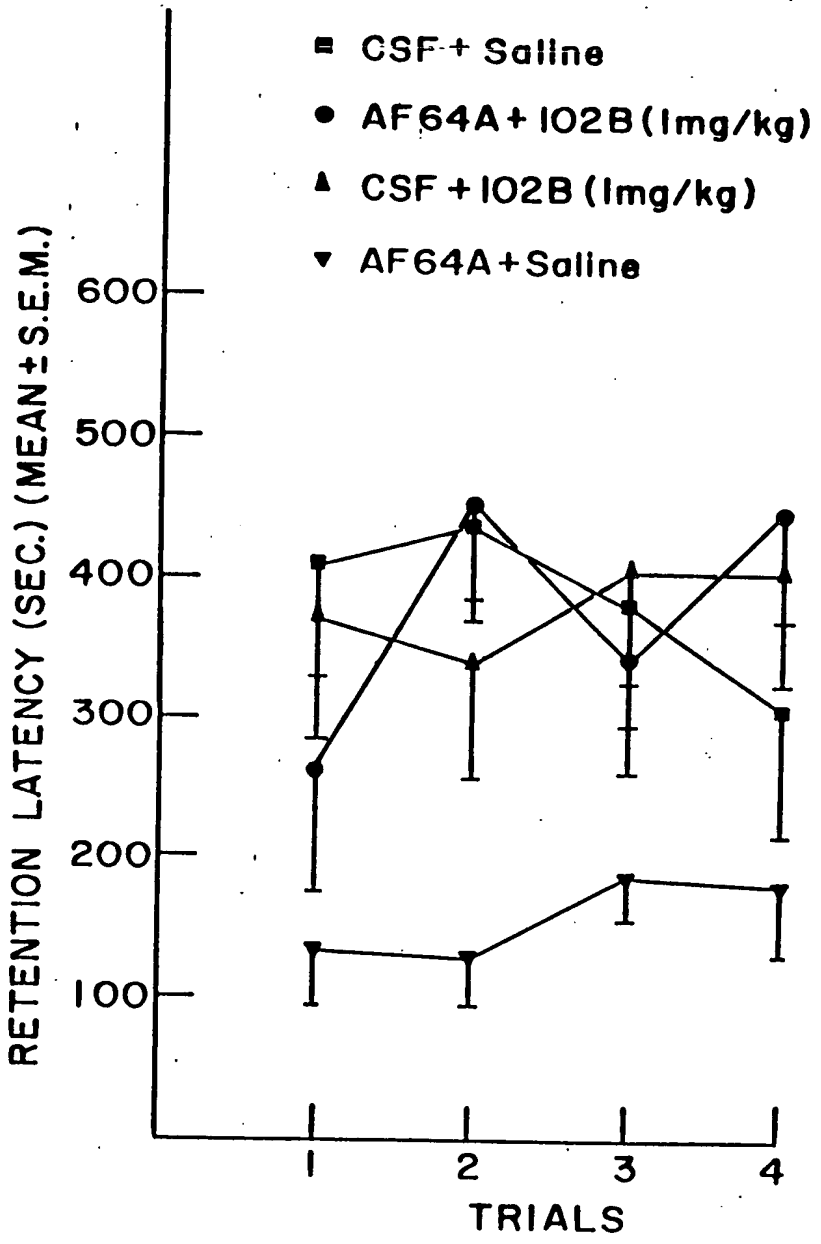


Fig. 9 : Retention-test latency measures during 4 trials of "extinction + latent extinction"

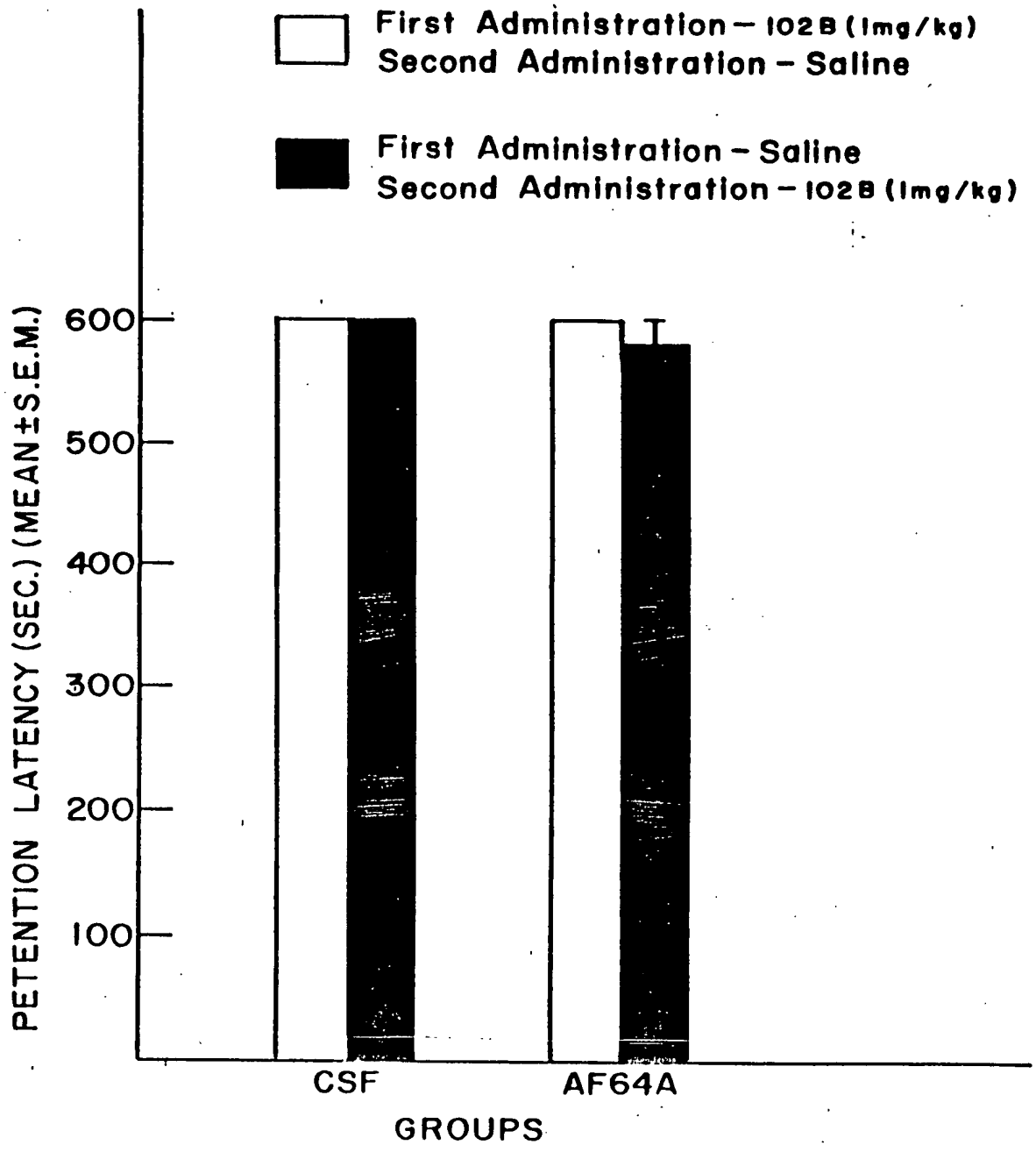


Fig. 10: Retention-test latency measures (sec.) of the AF64A and CSF-injected groups, after second administration of AF102B or saline.

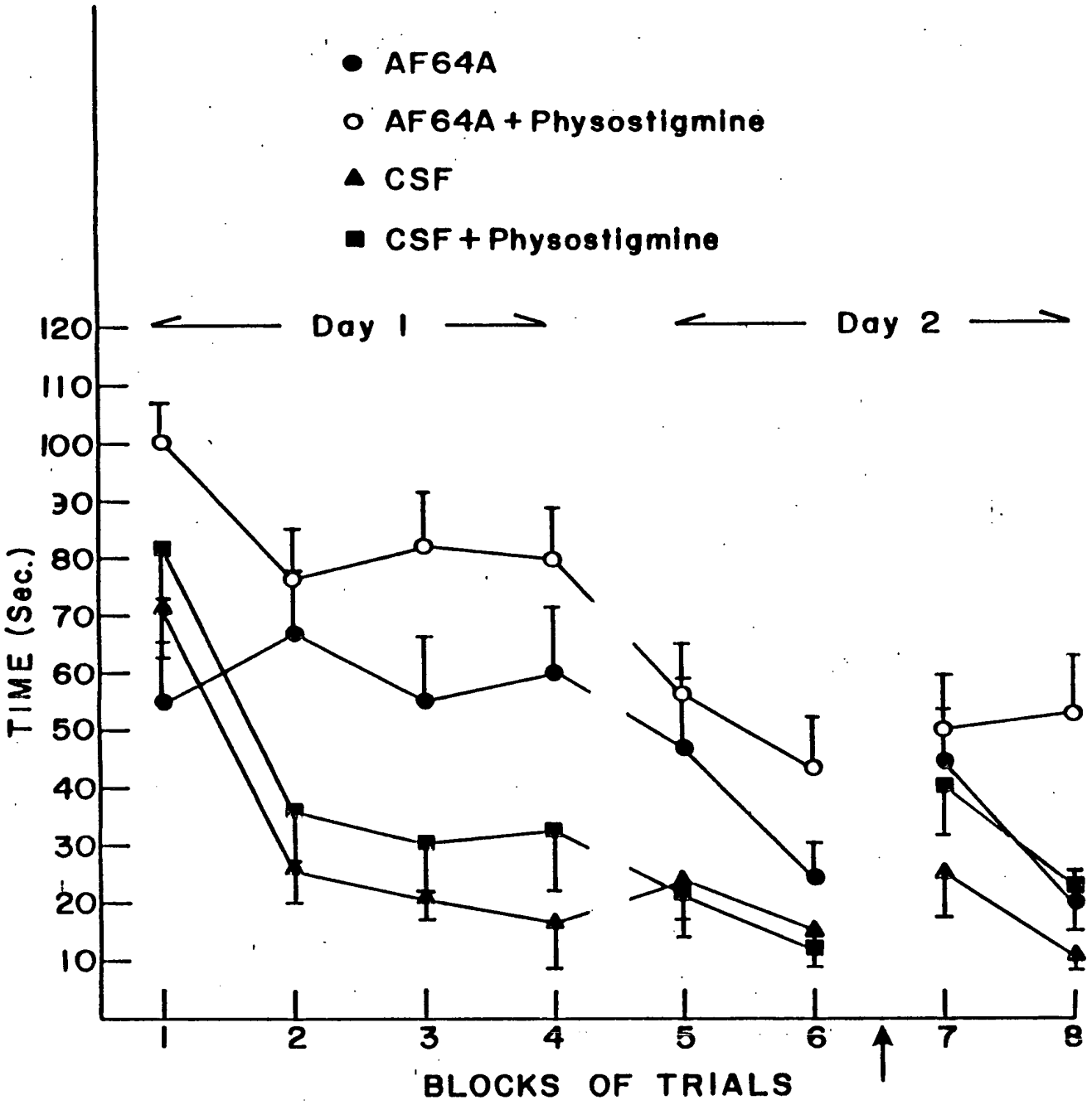


FIG 11: Escape latency measures, in blocks of 2 trials, of the AF64A and CSF-injected groups, after physostigmine administration.